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HISTOLOGY OF HUMAN YELLOW FEVER WHEN DEATH IS DELAYED

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Fatal cases of yellow fever usually terminate within ten days after onset. The criteria for the histologic diagnosis of the hepatic lesions in such cases have been well defined by the studies of Councilman,[1] Rocha-Lima,[2] Magarinos Torres,[3] Klotz and Belt [4] and others.

The aim of the present study is to describe the microscopic appearance of the liver in cases of yellow fever in which death is so delayed that the classic histologic picture is no longer found.

The classic picture of yellow fever in sections of human liver stained with hematoxylin and eosin (*A* in figure) consists of:

1. *A salt and pepper distribution throughout the entire lobule (but with greatest prevalence in the midzone) of rounded refractile acidophilic bodies, the so-called Councilman bodies.* These acidophilic bodies at times reveal the "shadows" of nuclei, and there can be no doubt that they are the remains of parenchymal cells which have undergone a special type of necrosis. (Similar necrotic cells may be produced by conditions other than yellow fever, but in such conditions their distribution within the lobule is generally quite different.)
2. *An absence of complete necrosis of the central zone of the liver lobule.* Even in cases in which the Councilman type of necrosis seems at first glance to involve the entire central zone a few nonnecrotic parenchymal cells can always be found among the cells immediately adjacent to the central vein.

The studies and observations on which this paper is based were made under the auspices of the Cooperative Yellow Fever Service maintained by the Ministry of Education and Health of Brazil and the International Health Division of the Rockefeller Foundation.

Dr. Fred L. Soper and Dr. J. A. Kerr offered valuable suggestions regarding this study. Numerous physicians of the Cooperative Yellow Fever Service of Brazil collaborated by securing the essential clinical data.

1. Councilman, W. T.: Report on the Etiology and Prevention of Yellow Fever, United States Treasury Department, United States Marine-Hospital Service, 1890, p. 151.
2. da Rocha-Lima, H.: Verhandl. d. deutsch. path. Gesellsch. **15**:163, 1912.
3. Magarinos Torres, C.: Mem. Inst. Oswaldo Cruz **25**:81, 1931.
4. Klotz, O., and Belt, T. H.: Am. J. Path. **6**:663, 1930.

3. A jumbling of the trabeculae, more accentuated in the midzone of the lobule than elsewhere.
4. A fatty degeneration of varying intensity but generally more abundant in the central and peripheral zones than in the midzones of the lobule.

Findings of secondary value in establishing a diagnosis include:

5. Nuclear changes, such as edema and intranuclear inclusions.[5]
6. Varying leukocytic infiltration, principally of mononuclear cells, most pronounced in the midzone.
7. Yellow pigment in the parenchymal cells of the central zone.
8. Hyperemia of the sinusoids, more pronounced in the midzone.

Among the liver tissues obtained by viscerotomy and examined in the laboratory of the Cooperative Yellow Fever Service at Rio de Janeiro, Brazil, are several on which a definite diagnosis of yellow fever can be made on the basis just outlined but which have, in addition to the usual findings in yellow fever liver, a varying number of unusual bright ochre-colored granular bodies interspersed among the Councilman bodies; the latter are quite typical but few. These granular bodies vary in size but are generally smaller than Councilman bodies. Though they may be found free among the non-necrotic parenchymal cells, they are more often found within the macrophages and Kupffer cells.

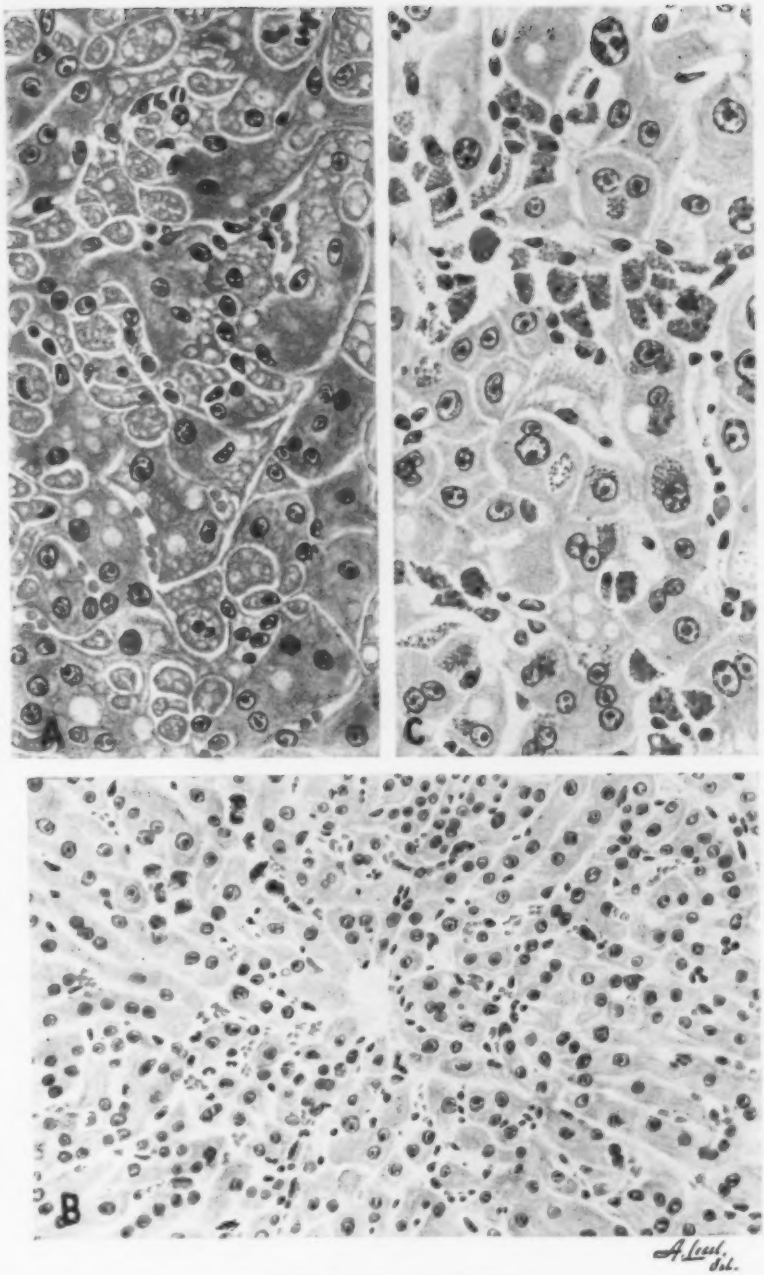
These granular bodies are:

1. Dirty yellow or ochre-colored in unstained frozen sections.[6]
2. Unchanged (ochre) in unstained preparations treated with hydrogen peroxide or with acid alcohol.
3. Orange ochre in preparations stained with sudan III.
4. Black in material stained by the del Río Hortega method (impregnated with silver carbonate and counterstained with gold chloride); the Councilman bodies are a dark violet.
5. Negative to the prussian blue test.

In addition, there are in the Cooperative Yellow Fever Service collection a number of tissues in which Councilman bodies either are not present (*B* and *C* in figure) or are present in such small numbers that a diagnosis of yellow fever cannot be made but which have these same bright ochre-colored granular bodies scattered salt and pepper

5. The intranuclear changes described by Magarinos Torres are of importance in the diagnosis of experimental yellow fever in rhesus monkeys but have not been of practical value in the routine examination of material collected through viscerotomy in South America.

6. All material received is fixed originally in 10 per cent formaldehyde in physiologic solution of sodium chloride.



EXPLANATION OF PLATE

A, classic picture of yellow fever in section of human liver stained with hematoxylin and eosin. Councilman bodies are distributed throughout, particularly in central zone, and there is absence of complete necrosis of the central zone.

B and *C*, sections of human liver in which the classic picture is not found but in which bright ochre-colored granular bodies are scattered throughout the lobule, with the greatest concentration in the midzone. These granular bodies have been used as a basis for the diagnosis of yellow fever in cases in which eosinophilic Councilman bodies could not be found.

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fashion throughout the entire lobule, with greatest prevalence in the midzone. The distribution of these bodies is then the same as that of the Councilman bodies in the classic yellow fever liver; by studying numerous preparations, it has been possible to follow the transition in the color of the Councilman bodies from the eosinophilic pink of the early stage of the disease to the bright ochre color; the Councilman body first loses its eosinophilia and becomes brownish yellow; this brownish yellow color becomes deeper in tone until it is bright ochre, as described.

It is believed that the color of the granular bodies results from the impregnation of the disintegrating Councilman bodies with bile pigment.

The presence of the ochre bodies has been used as a basis for the diagnosis of yellow fever in cases in which eosinophilic Councilman bodies could not be found.[7] In these cases other changes are found which support the diagnosis of yellow fever. They include:

1. Fatty degeneration, usually slight in degree and most often found in the central zone.
2. Slight jumbling of the trabeculae in the midzone.
3. Increase in size of the nuclei of the parenchymal cells.
4. Hyperplasia of the Kupffer cells and the presence of small groups of leukocytes, most frequently in the midzone.

Mitotic figures are extremely rare in the tissues studied. In 2 tissues [8] the midzones of many lobules have small areas of finely granular parenchymal cells with pale cytoplasm, in which mitotic figures are abundant. The parenchymal cells around the central veins and the periphery of the lobules show no mitosis and take a deeper stain, suggesting that these cells have survived. Klotz and Belt described [9] a similar condition present during the regeneration of hepatic tissue in rhesus monkeys recovering from experimental yellow fever.

COMMENT

The observations reported in the foregoing section have been made as the result of the study of a case in which a clinical diagnosis [10] of yellow fever had been made in the field on the basis of (1) classic symptoms and (2) the occurrence of other cases on the same fazenda

7. Generally these were cases in which death occurred late in the course of the disease.

8. Lab. no. 46.640, death on twelfth day; lab. no. 128.180, death on thirteenth day.

9. Klotz, O., and Belt, T. H.: Am. J. Path. **6**:689, 1930.

10. This was the case of patient M. S., of Mont Serrat, Rio de Janeiro, Brazil, February 1938, lab. no. 124.569. The original diagnosis was made by Dr. Ferreira Neves and was confirmed by Drs. Nicomedes de Almeida, D. B. Wilson and F. L. Soper.

in which the diagnosis was confirmed pathologically. On the eighth or ninth day after onset intercurrent bronchopneumonia became apparent, death occurring on the seventeenth day.

The liver tissue in this case was reported as negative for yellow fever. The refusal of the Cooperative Yellow Fever Service to accept this negative report in the face of the clinical findings led to a restudy of this and similar tissues, which resulted in the observations herewith presented.

Every laboratory material which had a histologic diagnosis at variance with the clinical diagnosis was reexamined, and in some cases it was possible to establish the diagnosis of yellow fever.[11] There are now 23 cases in which the diagnosis of yellow fever has been made in the absence of the classic alterations described for the yellow fever liver.

The distribution of these cases by reported duration of illness is as follows:

Days of Illness	Cases
8	1
9	2
10	3
11	4
12	2
13	5
14	0
15	4
16	1
17	1
Total	23

No clinical data are available regarding 4 cases, but in the remaining 19 the final histologic diagnosis agrees with the clinical data obtained by field investigation. One of the 19 cases is noteworthy because the clinical diagnosis was confirmed by the isolation of the yellow fever virus from blood serum secured on the second day of illness.[12]

SUMMARY

The classic picture of yellow fever in sections of human liver stained with hematoxylin and eosin is first given. Then 23 cases are reported in which a diagnosis of yellow fever was made in the absence of the typical microscopic picture found in the liver tissue of the patient who dies within ten days after the onset. In cases in which eosinophilic

11. Lab. nos. 46.640, 70.971, 71.621 and 74.192.

12. This was the case of A. R., of Muqui, Espirito Santo, Brazil, occurring in March 1939; death ensued on the eleventh day of illness. The tissue examined was Lab. no. 162.160.

Councilman bodies could not be found, the presence of bright ochre-colored granular bodies was used as a basis for the diagnosis of yellow fever (the color of these granular bodies is believed to result from the impregnation of the disintegrating Councilman bodies with bile pigment). To lend support to the diagnosis of yellow fever in these cases there were other changes, including fatty degeneration, slight jumbling of trabeculae in the midzone, an increase in the size of the nuclei of parenchymal cells, hyperplasia of Kupffer cells and the presence of small groups of leukocytes, also usually in the midzone. Mitotic figures were extremely rare in the tissues studied.

ABSORPTION OF LIQUID PETROLATUM ("MINERAL OIL") FROM THE INTESTINE

A HISTOLOGIC AND CHEMICAL STUDY

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The use of liquid petrolatum (medicinal "mineral oil") as a laxative for real or fancied intestinal stasis was popularized chiefly through the recommendations of Sir W. Arbuthnot Lane [1] in the first decade of the twentieth century. Liquid petrolatum is one of several products obtained from petroleum by distilling off the more volatile products, chilling, purification by filtration through bone, etc. According to the process of preparation, products of various consistencies are obtained, varying from waxy paraffin to the liquid mineral oil of household use. The latter consists of hydrocarbons of the methane and related aliphatic series; the medicinal product (liquid petrolatum) commonly used in this country consists chiefly of the saturated hydrocarbons. The earliest internal use of refined petrolatum probably dates to 1872, when Robert A. Chesebrough,[2] of New York, was granted a patent for the manufacture of "a new and useful product from petroleum, named vaseline." In the period prior to 1900 Randolph [3] and others [4] published occasional reports advocating the use of liquid petrolatum but it was not until this product was lauded in the widespread articles of Lane, supported by his position as chief surgeon of Guy's Hospital, that there was general acceptance. In 1914 the Council on Pharmacy and Chemistry of the American Medical Association [5] discussed liquid petrolatum and suggested standards for the various types of the oil. During the years since then the popularity of this product has been increasingly widespread until at the present time it is unusual to find any person who has not occasionally used liquid petrolatum.

From the Department of Pathology, University of Chicago.

1. Lane, W. A.: Brit. M. J. **2**:1125, 1913.

2. Chesebrough, R. A., cited by Council on Pharmacy and Chemistry.[5]

3. Randolph, N. A.: (*a*) Proc. Acad. Nat. Sc., Philadelphia (1884), 1885, p. 281; (*b*) Therap. Gaz. **9**:732, 1885

4. (*a*) Hutchison, R.: Brit. M. J. **1**:724, 1899. (*b*) Robinson, W. D.: M. News, New York **77**:55, 1900.

5. Liquid Petrolatum or "Russian Mineral Oil," report of the Council on Pharmacy and Chemistry, J. A. M. A. **62**:1740, 1914.

From the first the question of the absorption of the oil was of interest. Experimental work designed to determine whether the material passed in toto through the intestinal canal or was partially taken into the body was done on human subjects and on animals. Such experiments were of two main types: The first consisted of feeding a certain amount of liquid petrolatum to the subject and then determining how much could be recovered from the feces or from the lymph stream containing ingested products. The second method, used chiefly by later workers, involved a search in the tissues of the animal body for ingested hydrocarbon after the latter had been fed to the animal. In general, the results from the first method were interpreted to indicate that liquid petrolatum was not absorbed, and the results from the second, that some absorption probably did occur.

REVIEW OF THE LITERATURE

The first recorded attempts to determine experimentally the absorption of liquid petrolatum were reported by Randolph,[3a] in 1884. He fed 2 healthy adults 30 cc. of "vaseline" (probably petrolatum) over a period of forty-eight hours. The alvine discharges for the next three days were collected, dried and extracted with purified petroleum benzine (petroleum ether). "Making a slight allowance for incompleteness in extraction, the vaseline ingested was in each case recovered in its totality, showing that it has passed through the economy unchanged and unabsorbed." In 1889 Hutchison [4a] published similar results. He fed a human subject 7 Gm. of liquid petrolatum and recovered 7.2 Gm. of unsaponifiable material. He concluded that the excess was probably due to traces of soaps. One year later Robinson [4b] made the following assertion: "That every minim swallowed passed unchanged entirely through the intestinal tract of man was repeatedly proven. Nature evidently made no effort towards its digestion or assimilation." No details of the work by which these conclusions were reached were given. In the same year a report of the first experimental work in which animals were used was published by Henriques and Hausen.[6] An emulsifiable mixture of petrolatum and lard was fed to rats, and 98 per cent of the liquid petrolatum was recovered from the feces. From this the conclusion was drawn that liquid petrolatum could not be absorbed. Continuing the use of human adults as experimental subjects, Schmidt [7] fed up to 20 Gm. of liquid petrolatum at one time without observing any injurious effect. He was unable to recover from the feces the entire quantity of the product administered and believed that a certain portion of it, probably the fractions with low boiling points, were absorbed or possibly oxidized in the organism.

The most detailed experiments of this first type (analysis of the feces for material fed) were reported by Bloor [8] as part of his studies on the absorption of fats. In his first group of experiments, solutions of unemulsified hydrocarbon oils were fed to cats and the amount of unabsorbed oils determined by extraction of the feces. The amount of recovered material ranged from 85.5 to 102.5 per cent of

6. Henriques, V., and Hausen, C.: Centralbl. f. Physiol. **14**:313, 1900.
7. Schmidt, A.: München. med. Wchnschr. **52**:1970, 1905.
8. Bloor, W. R.: J. Biol. Chem. **15**:105, 1913.

the amount fed. His conclusions were: "The recovery of the hydrocarbon jelly was practically complete as was also that of the hydrocarbon oil when fed alone, while there was a slight discrepancy in the recovery of the hydrocarbon oil when fed in solution in olive and cocoanut oils which may have been due to absorption. Taking into account, however, the error of the experiment, the amount absorbed was probably negligible. The possibility of absorption of these substances either in solution in fats or fatty acids or in the form of emulsions is shown to be extremely unlikely."

Another general method of experimental approach used in this problem consisted, as has been stated, in the study of the contents of the lymphatics draining the intestines after the hydrocarbon has been fed. Bradley and Gasser [9] fed a dog an emulsified mixture of olive oil and liquid petrolatum and found in the thoracic lymph both oils in about the same relative proportion as in the emulsion which had been fed. This suggested to them a mechanical absorption of droplets of mixtures of fatty acid and hydrocarbon oil. Bloor [8] repeated these experiments but with opposite results. The unsaponifiable material which he recovered from the chyle fat was believed to be chiefly cholesterol, and he concluded that "the amount and nature of the unsaponifiable matter precludes the possibility of the absorption of any appreciable amount of the hydrocarbon oil. The results . . . give no grounds for the belief that these substances are absorbed from the intestine."

Gage and Fish [10] studied the digestion, absorption and assimilation of fat in man and animals by observing fat particles ("chylomicrons") in the circulating blood by means of the dark field microscope and a fat-soluble dye. Testing the digestibility of castor oil and liquid petrolatum (on man?), they found that "there was no increase in the chylomicrons, and we interpret the findings to mean that no digestion of either substance took place. There was no purgative action until after the actual period for the digestion of fatty food; therefore, both the substances were in the intestine a sufficient time for thorough digestion. When mixed with butter, the mineral oil is left behind but the butter fat is picked out, digested and absorbed." Mellanby [11] carried on experiments similar to those of Bloor but used bile as the emulsifying agent. The cat was used as the experimental animal. Examination of the intestine and mesentery during a period of six hours showed that there was no absorption of petroleum into the lymphatic system of the intestine, although the oil passed rapidly down the intestine. The method of examination was not reported.

Both of the general methods described are of value only for indicating absorption of a large proportion of ingested oil. For study of the possible absorption of small amounts, more delicate methods are necessary. The experiments of Channon and Collinson [12] were of this type. These workers studied the unsaponifiable fractions of the livers of animals which had received liquid petrolatum in their diet, comparing the iodine number of this fraction with that of the livers of animals receiving the control diet only. Rats and swine were used as the experimental animals, and similar results were attained with each. The rats were maintained on a diet containing 5 per cent liquid petrolatum for five weeks; the yield of unsaponifiable matter from the livers of the animals which had received the oil was increased by 40 per cent over that of the control groups, while the iodine

9. Bradley, H. C., and Gasser, H. S.: J. Biol. Chem. **11**:xx, 1912.
10. Gage, S. H., and Fish, P. A.: Am. J. Anat. **34**:1, 1924.
11. Mellanby, J.: J. Physiol. **64**:xxxiii, 1927.
12. Channon, H. J., and Collinson, G. A.: Biochem. J. **23**:676, 1929.

value of the nonsterol portion (cholesterol removed by digitonin) was 30.8 in the group fed oil and 118.8 in the control group. Two pigs were used: One was given the control diet and the other the same diet plus 100 cc. daily of liquid petrolatum emulsified with acacia. The animals were killed after fifty-four days. The comparative iodine values here were 36.6 for the anmial fed the oil and 112.8 for the control animal. Other determinations (acetyl value, percentage of bromine in the brominated product) gave similar results. In a discussion of the possible mechanism by which the oil was absorbed, the authors suggested that in spite of much contrary evidence particulate absorption may occur.

Twort and Twort[13] reported the first histologic changes thought to be due to hydrocarbons. After application of liquid petrolatum (the product was called "mineral oil" in the article but in a subsequent report was designated as "medical liquid paraffin") to the skin of mice, the livers were found to show first periportal infiltration by small round cells; the cells later became larger, with eccentric nuclei, called by them "endotheloid-xanthomatoid." These cells underwent disintegration with formation of foamy cytoplasm and later coarse vacuolation with ultimate replacement by a globule of fat. Whether this fat was of a saponifiable or a hydrocarbon nature could not be stated. Similar cells were occasionally seen in the spleen, adrenals and ovary. The authors believed that the oil had access to the internal organs by the alimentary tract, with the animals ingesting the oil from the skin. The condition developed also in rabbits and rats. No further reports have appeared to indicate their conclusions as to the nature of the condition. Tantini[14] published the results of experiments in which he fed liquid petrolatum by mouth to guinea pigs for a four month period. He stated that the oil could be demonstrated in the hepatic cells, where it could be differentiated from the other fat by the Carminati method. The oil was said to produce lesions of a regressive nature in these cells, distributed focally, with a preference for the centers of lobules. No lesions were seen in the intestine or in other organs studied (pancreas, spleen). An example of histopathologic change in man was reported by Jaffé.[15] Histologic examination of an appendix removed from a woman with the symptoms of acute appendicitis showed tissue similar to that seen in paraffinoma, including giant cells. The contents of the cells stained red with scarlet red, and chemical analysis was said to reveal liquid petrolatum. No further details were given.

It is the current practice to teach that liquid petrolatum is not absorbed from the intestine. Thus the textbook of pharmacology by Sollmann[16] states "Mineral oils are not absorbed (except perhaps doubtful traces: Bradley and Gasser, 1912), but are eliminated unchanged in the feces (Randolph, 1885; Hutchison, 1899; Bloor, 1913)." Similar statements have been made by Cushny,[17] Clark,[18] Bastedo,[19]

13. Twort, C. C., and Twort, J. M.: Lancet **1**:448, 1932; J. Hyg. **33**:396, 1933.

14. Tantini, E.: Tumori **9**:266, 1935.

15. Jaffé, R. H.: Deutsche med. Wchnschr. **60**:508, 1934.

16. Sollmann, T.: A Manual of Pharmacology, ed. 5, Philadelphia, W. B. Saunders Company, 1936.

17. Cushny, A.: A Textbook of Pharmacology and Therapeutics, ed. 11, Philadelphia, Lea & Febiger, 1936.

18. Clark, A. J.: Applied Pharmacology, ed. 6, Philadelphia, P. Blakiston's Son & Co., 1938.

19. Bastedo, W. A.: Materia Medica, Pharmacology and Therapeutics, ed. 3, Philadelphia, W. B. Saunders Company, 1932.

McGuigan [20] and Beckman [21] and in "Useful Drugs." [22] In 1937 the Council on Foods of the American Medical Association,[23] in a discussion of the use of mineral oil in foods, stated, "It is well known that liquid petrolatum is not absorbed from the gastrointestinal tract," but the statement was qualified by a footnote calling attention to the work of Channon and Collinson. The results just reviewed seem to indicate that such qualifications are necessary. While the earlier types of experimentation did indicate that there was no marked absorption of the hydrocarbons, the methods were not of use in detecting minimal absorption. For this, experiments similar to those of Channon and Collinson plus histologic studies of the type described by Twort and Twort and by Tantini are necessary.

STATEMENT OF THE PROBLEM

In the routine autopsy studies of this laboratory lesions have been observed in various tissues which it was thought might be due to the ingestion of liquid petrolatum. The present investigation was devised to determine whether absorption of this oil occurs, and, if so, what type of lesions result.

MATERIAL AND METHODS

For the experimental part of the problem rabbits, rats and guinea pigs were used. Four brands of liquid petrolatum were employed: 1. Heavy white mineral oil (Sonneborn), from Pennsylvania fields. This oil is stated to be completely saturated; it has a specific gravity of 0.875 to 0.885. 2. Light white mineral oil (Sonneborn), similar in composition to the heavy type, but with a specific gravity of 0.860 to 0.870. 3. McKesson's albolene. 4. Russian mineral oil (Walgreen Drug Stores). No differences were noted to depend on the type of oil used. When the animals died or were killed after selected intervals of time, complete autopsies were done. In every case blocks of the intestine, mesenteric lymph nodes, liver and spleen were fixed in Zenker's solution, and sections made and stained with hematoxylin and eosin. In addition, in some instances, histologic examinations of other organs were made. In many of the discussions of pneumonia due to liquid petrolatum, the failure of reduction of osmic acid has been considered a specific reaction to indicate the type of oil present. While it is true that this method would differentiate liquid petrolatum from animal or vegetable neutral fats and fatty acids, it would not prove the absence of cholesterol, phosphatides or cerebrosides, none of which reduce the acid. These are the lipids characteristic of the lipoid storage diseases, and thus a means of differentiation from them is essential. Further, it has been shown [24] that the use of osmic acid on tissue fixed in solution of formaldehyde U. S. P. is likely to lead to false interpretations, since after such fixation certain lipoprotein combinations often reduce the acid. However, the stain is of corroborative value, and as such it has been used. For the specific

20. McGuigan, H. A.: Applied Pharmacology, St. Louis, C. V. Mosby Company, 1940.

21. Beckman, H.: Treatment in General Practice, ed. 3, Philadelphia, W. B. Saunders Company, 1938.

22. Useful Drugs, ed. 11, Chicago, American Medical Association, 1938.

23. Mineral Oil in Foods, report of the Council on Foods, J. A. M. A. **109**: 1814, 1937.

24. Hoerr, N. L.: Anat. Rec. **66**:149, 1936.

detection of liquid petrolatum in tissues, the method of Carminati [25] has been recommended. This stain is based on a difference in the solubility of orange sudan G in liquid petrolatum as compared with other lipids or oils. The results obtained with this method, however, are not completely satisfactory. If no sudanophilic material other than liquid petrolatum is present, a good contrast may be achieved between the oil, staining yellow, and the surrounding tissues. However, normal saponifiable fats, as in adipose tissue, or pathologic fats, as in fatty metamorphosis in the liver, may with the Carminati method stain either orange or yellow or in some cases may stain yellow centrally and orange peripherally. Further, while the stain may be of some value if the material stained is in large drops, when it is in small droplets or in cells there is insufficiently sharp differentiation. This difficulty is especially noted in sections of the liver where the hepatic cells and the red blood cells also stain yellow with orange sudan G. Thus the stain was found to be of most value in the differential staining of intestinal mucosa and mesenteric lymph nodes and was of less value in the examination of the liver and spleen unless the liquid petrolatum was in relatively sharply demarcated aggregations of macrophages. Where the differential staining by the Carminati method was most marked, the sudan IV (scarlet red) stain likewise showed differences between the staining of saponifiable fats and that of liquid petrolatum, the latter being light orange and the former dark orange. There is the possibility that this difference depends on the quantity of material present to be stained. All the specific fat stains were used on gelatin-embedded material.

The macrophages containing liquid petrolatum have a characteristic appearance, which was found to be nearly as valuable a means of identification as the stains used. There is marked variance in the size of adjacent droplets. Some are definitely intracellular but even here variable in size; there is not the homogeneous appearance of the alimentary lipophages, of the fatty macrophages of the lipoid storage diseases or of the brown fat of the lower animals, nor are there the single large drops of neutral fat found in the normal mature fat cell. Many of the drops of oil appear to be extracellular, and giant cells of the type often found as a reaction to a foreign body are frequently seen about them. In all the tissues the oleophages tend to occur in aggregations, but isolated cells are also seen. It will be noted that this appearance is characteristic of the reaction to liquid petrolatum in other tissues, such as the lungs in lipid pneumonia, the subcutaneous tissues in paraffinoma, the pleura following oleothorax due to the injection of liquid petrolatum and the peritoneum in the reaction following the intraperitoneal injection of liquid petrolatum.

In the microscopic examination of the tissues of the animals and of the autopsy material from human subjects a comparative grading scheme was adopted with values of + to ++++, depending on the quantities of oleophages and extracellular oil present.

Chemical analyses of the tissues were made in an attempt to recover any liquid petrolatum that might be present in the various organs. The microscopic examination would show the location of any ingested hydrocarbon, while the chemical analysis would offer supplementary but more definite proof of the presence of the substance. These analyses were of tissues from the experimental animals and from control animals of similar ages. The unsaponifiable fats and oils were determined, the saturated hydrocarbons isolated and a comparison made of the amounts of material thus obtained from experimental and control animals. A more detailed description of the process is given later.

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25. Carminati, V.: Sperimentale, Arch. di biol. **88**:564, 1934.

In addition to this experimental work, autopsy material was studied to determine whether any absorption of liquid petrolatum occurred in the human subject. Sections of the intestines, mesenteric lymph nodes, liver and spleen were examined in such cases as they were available. Routine sections of the intestines, mesenteric lymph nodes, liver and spleen were also studied from 25 consecutive autopsies of bodies over 21 years of age at Albert Merritt Billings Hospital regardless of the history. Microscopic examinations and chemical analyses of these tissues were made when indicated and possible.

DETAILS OF EXPERIMENTS

Thirteen adult white rabbits, 21 albino rats and 31 guinea pigs were fed varying amounts of liquid petrolatum.

The rabbits were fed the regular stock laboratory diet consisting of Alpha flakes [26] with frequent supplements of carrots and lettuce. The chosen amount of liquid petrolatum to be given the animal was poured over the top of the flakes; the animals ate these oil-covered flakes as readily as the dry flakes. The dosage of oil was 20 cc. or 30 cc. daily; the duration of the feeding varied, with the longest period thirteen months.

Two groups of rats were used. The fifteen animals of the first group were fed white bread over which liquid petrolatum had been poured. Supplements of carrots and lettuce were given. This method of feeding did not permit measurement of the quantity of oil ingested by each rat. The duration of feeding of the rats varied up to nineteen months. The 12 rats of the second group were divided into two subgroups of 6 animals each. Each subgroup was fed the stock laboratory breeding diet, but 5 per cent of light liquid petrolatum was added to the food of one subgroup. The rats were fed their respective diets for six weeks and then were killed. The livers of these animals were chemically analyzed; this portion of the problem was a repetition of the work of Channon and Collinson.

The guinea pigs were fed the same diet as were the rabbits. The animals received the chosen amount of liquid petrolatum by means of a 1 cc. tuberculin syringe; the glass end of the syringe was placed in the animal's mouth and as large a quantity of oil as the animal would swallow at one time was delivered. The quantity of oil fed the guinea pigs varied from 1 cc. to 20 cc. daily; the duration of the feeding ranged up to fifteen months.

Most of the animals were killed and examined within a few days after the final feeding of the liquid petrolatum.

RESULTS OF EXPERIMENTS

Gross Findings.—At autopsy the rabbits fed the oil for periods of seven months or less did not show gross changes that could be ascribed to the liquid petrolatum. The animals fed a longer time had in their intestines numerous 1 mm.-sized white nodules, elevated about 1 mm. above the surrounding mucosa; these were most conspicuous in the jejunum and the first portion of the colon. The mesenteric lymph nodes were enlarged, and on their surfaces were similar small white nodules. An occasional nodule could be seen in the liver.

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26. The formula for alpha flakes is given by the manufacturer as: protein not less than 15 per cent, fat not less than 3 per cent, fiber 12 per cent, carbohydrate 62 per cent, alfalfa, oats, barley, corn, soybean meal, whole wheat, dry buttermilk, linseed oil meal, salt, calcium carbonate and calcium sulfate.

Rats fed liquid petrolatum longer than seven months showed a similar involvement of their mesenteric lymph nodes. In their livers were numerous yellow flecks, which appeared to be distributed according to the location of the portal triads. Lesions were not seen in the intestines.

The guinea pigs did not have gross changes in any of their tissues.

Microscopic Observations.—Intestines: Changes considered to be the result of the feeding of liquid petrolatum were found in the intestines of the rabbits; such changes were not seen in the intestines of the rats or the guinea pigs. The lesions found in the rabbits were essentially of the same type in all the animals, but their extent increased with the length of time liquid petrolatum had been fed. If an animal had been fed this oil for a comparatively short time (e. g., four months), the vacuolated cells were seen in the lamina propria, most abundant in the jejunum but present also in the colon. The intracellular vacuoles varied in size, but most of them were comparatively large; occasionally the large ones were surrounded by smaller vacuoles. There was a tendency for several of the vacuoles to be in apposition. The larger number of these oleophages were in the superficial lamina propria near the tips of the villi in the jejunum, but occasional vacuoles were present in the deeper portions. If an animal had been fed the oil for a longer time (e. g., thirteen months), the sections of the jejunum, ileum and colon showed vacuolated cells in the same locations but much more numerous. In addition, interspersed among these cells were larger vacuoles that appeared to be extracellular and often were bordered by giant cells. In some sections of jejunum, the entire lamina propria at the tip of the villus was replaced by these two types. In the ileum, similar cells and vacuoles were seen in the intestinal lymphatic follicles.

Sections of intestine stained with sudan IV stain for fat showed sudanophilic material in those areas in which the vacuoles and vacuolated cells were seen in the sections stained with hematoxylin and eosin. The material stained a light orange in contrast to the orange-red color of the adjacent adipose tissue. With osmic acid, this material in the mucosa showed no reduction, while the adipose tissue was black. With the Carminati stain, the mucosal material was stained yellow, while the adipose tissue was orange.

Mesenteric Lymph Nodes: All three species of animals used had intracellular and extracellular vacuoles in their mesenteric lymph nodes. The extent of the lesions was again related to the length of time the liquid petrolatum had been fed. The earliest change seen in the nodes of the rabbits was the appearance in nearly every medullary cord of accumulations of large and small vacuoles, both intracellular and extracellular. These were similar to those seen in the intestine but were more numerous than in the intestine of the same animal and showed more variation in size. About some of the vacuoles an elongated

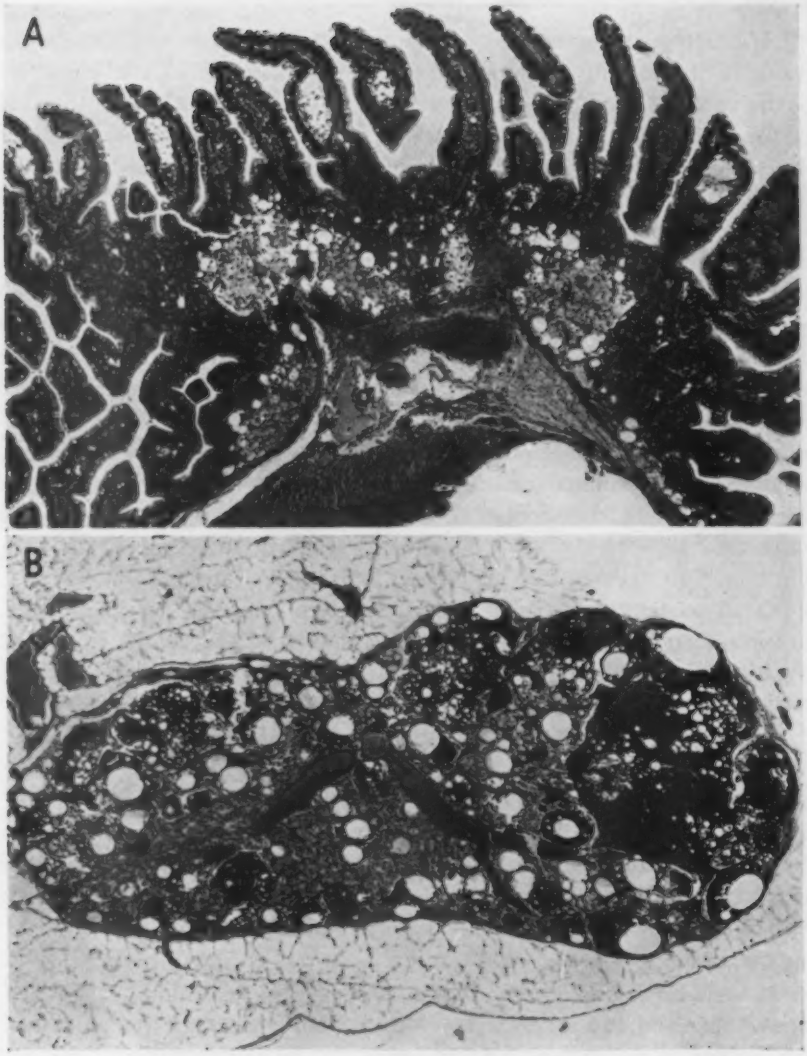

Fig. 1.—*A*, jejunum of rabbit fed liquid petrolatum for thirteen months. Numerous oleophages and extracellular collections of the oil are in the lamina propria. × 44. *B*, mesenteric lymph node of the same rabbit. The oil is located in the follicles and medullary cords. × 20.5.

crescentic nucleus could be seen. In these early stages only an occasional oleophage or extracellular vacuole was seen in the large peripheral mass of lymphatic tissue. No giant cells were found about the larger central vacuoles. In animals fed a longer period, however, poorly formed giant cells were present. In these nodes nearly all the lymphatic follicles, as well as the medullary cords, contained large numbers of the vacuolated cells and the extracellular vacuoles. The infiltration of the follicles and cords had distended those structures and placed the walls of the sinuses in apposition so that the outlines of the sinuses were difficult to ascertain.

The mesenteric lymph nodes of the rats showed similar changes, again varying according to the length of time the animal had received the oil. Giant cells were not seen. In the mesenteric lymph nodes of those rats of the second group which had been fed liquid petrolatum for only six weeks, a few scattered vacuolated cells were seen in the medullary cords; in the peripheral follicles of some of the nodes there were scattered nests of mononuclear cells with nonvacuolated eosinophilic cytoplasm. In the nodes of animals fed for nineteen months the intracellular and extracellular vacuoles were so numerous that in many places they had displaced nearly all the lymphatic tissue, so that only a small ring of lymphocytes outlined the former site of the cord or follicle. In other parts large numbers of lymphocytes remained.

The tissues of guinea pigs fed liquid petrolatum for a period of less than seven months did not show lesions. The mesenteric lymph nodes of some of the animals fed a longer time had occasional vacuolated cells and extracellular vacuoles in the medullary cords and peripheral follicles. These lesions had the morphologic appearance of those described in the lymph nodes of the rabbits and the rats.

Frozen sections of the mesenteric lymph nodes of all three species of animals were stained for fat. The results of the use of osmic acid and orange sudan G were not unequivocal in all sections, but the largest part of the sudanophilic material in the vacuolated cells and extracellular vacuoles did not reduce osmic acid and was yellow with the Carminati stain.

Liver: Lesions appeared in the livers later than in the lymph nodes. As in the other tissues, the extent of the lesions was related to the length of time the oil had been fed to the animal. It is possible that some of the earliest lesions were not recognized, because of a lack of clear differentiation from other pathologic changes in the hepatic tissue. The first lesion noted was the appearance adjacent to a few of the triads of occasional isolated vacuolated cells, in which the vacuoles varied in size; these cells resembled the cells in the intestines and mesenteric lymph nodes. The cells were neither numerous nor conspicuous. In the liver of a rabbit fed for a long time numerous vacuolated cells were scattered

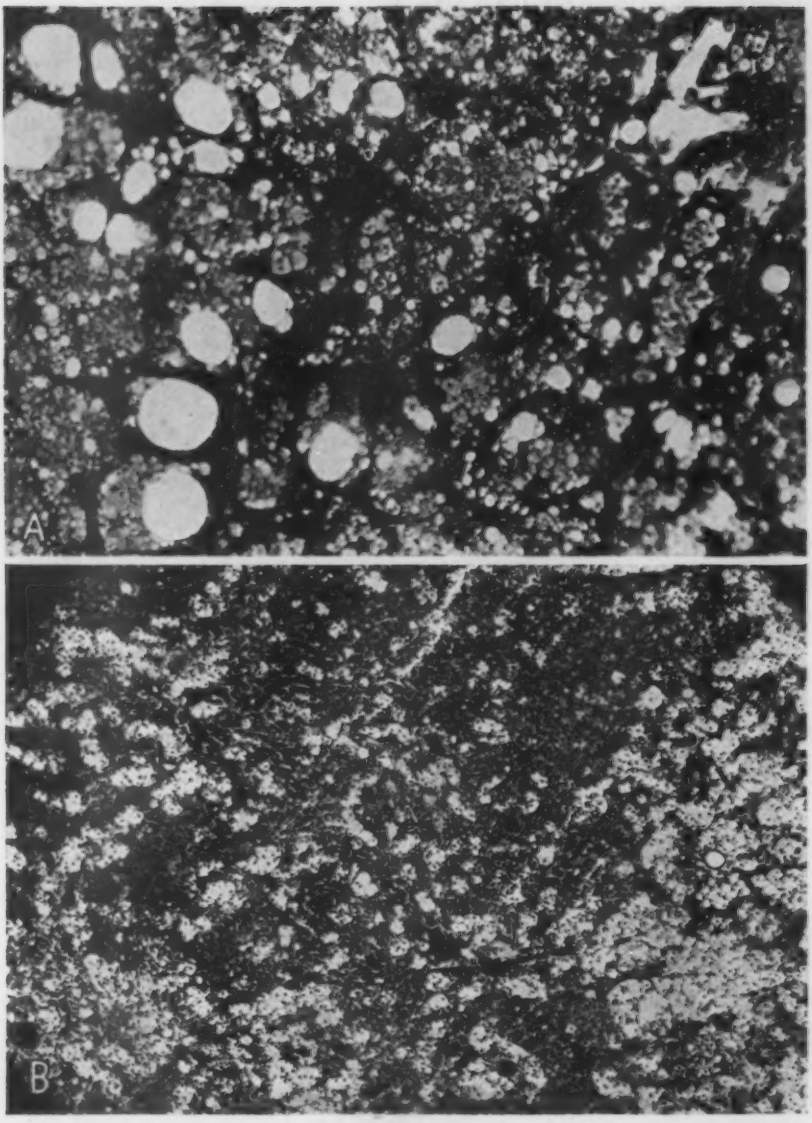

Fig. 2.—*A*, mesenteric lymph node of a rat fed liquid petrolatum for nineteen months. × 97. *B*, liver of the same rat. The macrophages containing the oil are located throughout the lobules; the majority are periportal. × 75.

irregularly throughout the lobules but most abundantly in the periportal areas. About some of these were small collections of lymphatic cells. These oleophages were again similar in structure to those seen in the intestines and in the mesenteric lymph nodes; they were not as well differentiated from the surrounding parenchymatous cells as were the similar cells in the other tissues.

In rats fed for a sufficient period the lesions were similar to those in the rabbit but more marked. Practically every triad was surrounded by large and small aggregations of vacuolated cells and extracellular vacuoles. These were both in the tissue of the triad and adjacent to the triads, where they had apparently replaced the liver parenchyma. Similar individual cells were scattered irregularly throughout the more central portions of the lobules. Giant cells were not seen.

In some of the sections of the livers of the guinea pigs a few vacuolated cells were seen adjacent to triads. In frozen sections the content of these cells appeared to be yellow with the Carminati stain, but there was insufficient contrast with the other tissues for evaluation. Since most of the livers of the guinea pigs showed marked fatty metamorphosis, no certain microscopic demonstration of liquid petrolatum was possible.

In many of the frozen sections of the livers of the rabbits and rats which were stained for fat no evaluation of the sudanophilic material present could be made by use of osmic acid or orange sudan G; the amount of material present was insufficient for differentiation. Where a large amount was present, however, globules corresponding to the vacuoles considered to indicate liquid petrolatum in the hematoxylin-eosin sections failed to reduce osmic acid and were yellow when stained by the Carminati method.

Other Tissues: Only one other tissue contained vacuolated cells. In the spleen of a rat fed for a long period many scattered small nests of oleophages were seen. All vacuoles here were intracellular. Many of these nests were definitely in the malpighian bodies; others were in the vicinity of arteries and trabeculae and may have been in the sites of former follicles from which the lymphocytes had been displaced. The material in the cells in the frozen sections did not reduce osmic acid and was yellow with the Carminati stain. Sections of the spleens of the rabbits and guinea pigs did not show such changes.

Other Lesions: Two other lesions of note were found. Leukemic infiltrations were present in all tissues examined from a rat that had been fed for a comparatively long period. Grossly, this leukemic tissue was green. In some of the guinea pigs fed over a long period there was infiltration of the submucosa and muscular coats of the intestines by vacuolated cells resembling adipose tissue; in frozen sections the con-

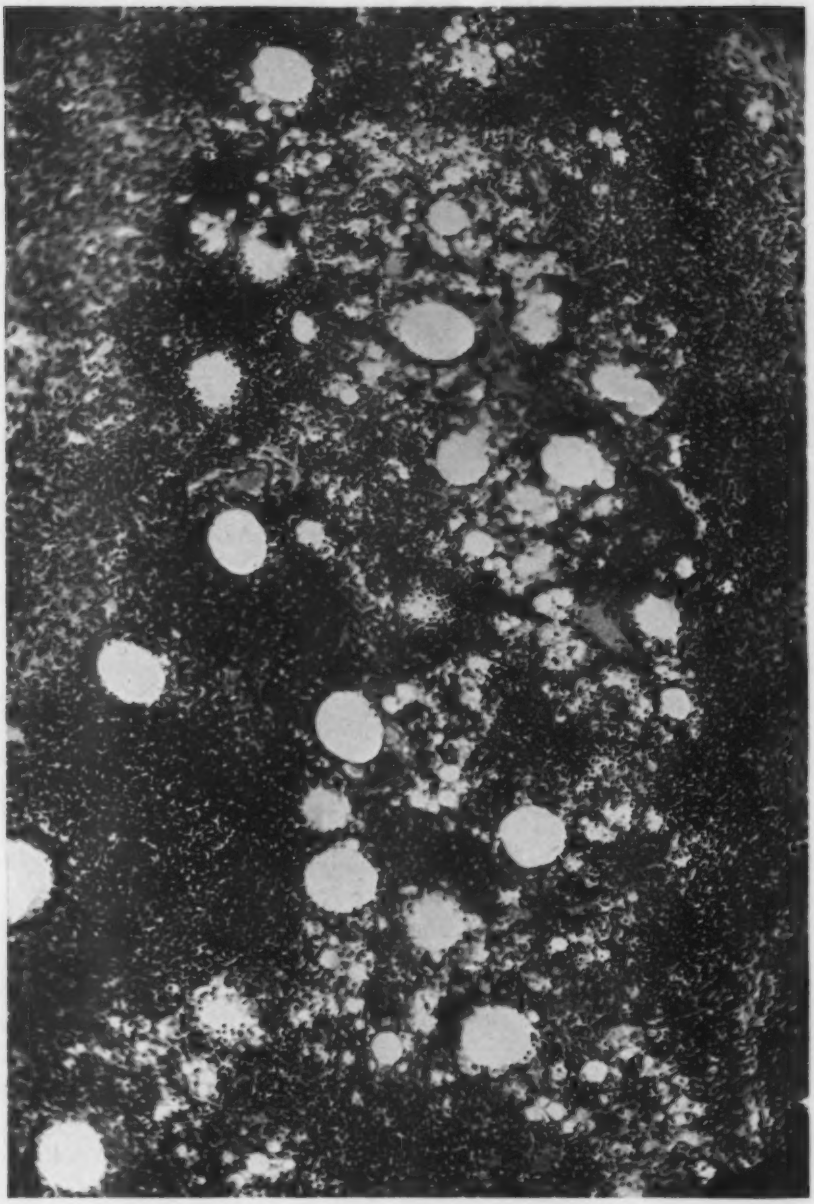

Fig. 3.—Mesenteric lymph nodes in case 2. There was a clinical history of the use of liquid petrolatum. Note the giant cell with vacuolated cytoplasm. × 110.

tents of these cells reduced osmic acid and stained orange with the Carminati stain. All other pathologic changes were those frequently seen in the three species of animals used.

Comment.—Although the absorption of liquid petrolatum was demonstrable in all three groups of animals used, there were interesting differences. In the rabbits intestinal lesions were a conspicuous feature; such lesions were absent in the rats and guinea pigs. Giant cells were seen only in the rabbit; the oil was seen in the spleen only in the rat. In all animals the ease of the demonstration of the oil and the quantity present were related to the length of time the material had been fed.

The evaluation of changes was more difficult in the case of the guinea pig than in that of the rabbit or that of the rat. It would appear that the oil was not absorbed as rapidly. This may have been related to the different method of feeding; the oil was administered in one dose daily, separate from the food. It was impossible to train the guinea pigs to eat a diet in which the oil and the food had been mixed. When other changes in the liver were absent, material appearing to give the staining reactions for liquid petrolatum could be shown, but the fat stains were of no value in the presence of generalized fatty metamorphosis. In general, in all the animals staining methods were of value only when relatively large quantities of oil were present.

It was also noted in the work with guinea pigs that not only was a sufficient period required before changes could be noted but also a relatively large quantity of the oil was necessary. The absorption was related to both factors; certain animals fed comparatively small amounts showed no changes although they were fed longer than six months, while 1 animal was fed 1,500 cc. of oil in six months without microscopic evidence of change.

HUMAN AUTOPSY MATERIAL

The material which was studied in order to observe the incidence of typical oleophages in human tissues was either the routine autopsy material of the department of pathology of the University of Chicago or special material taken at routine autopsies performed by members of the department. Sections of mesenteric lymph nodes are usually not made unless the prosector feels that there is some definite indication for the microscopic examination, and thus such sections were available in less than 15 per cent of the cases. In a series of slightly over 400 autopsies, however, there were at least 23 instances in which oleophages considered to be typical of the absorption of liquid petrolatum were present in the mesenteric lymph nodes. In 7 of these cases similar vacuolated cells were found also in the liver and spleen. Since sections of the intestines are taken for microscopic examination only when such study seems indicated at the time of autopsy, intestinal material was available in only 12 of the 23 cases. Oleophages were found in 1 of these 12 cases. In

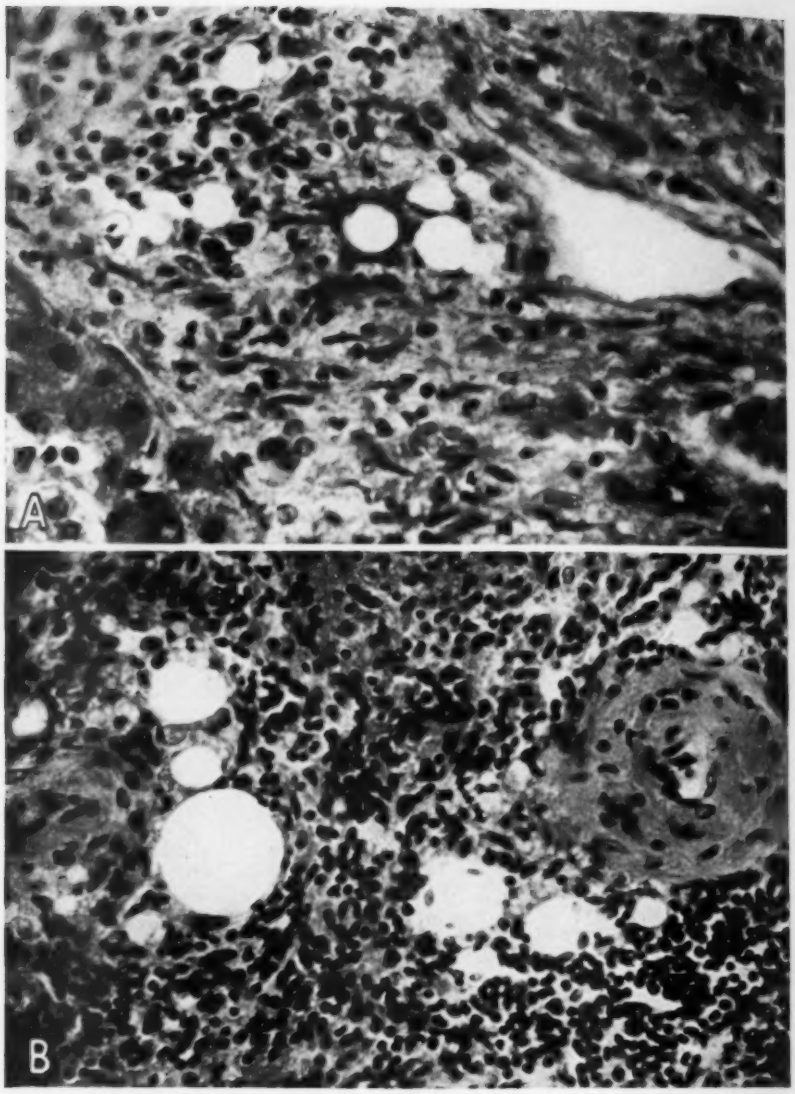

Fig. 4.—*A*, portal triad of a patient who had suffered from constipation for many years and had used numerous cathartics. Oil is present both in macrophages and in extracellular collections; lymphatic cells are adjacent. × 459. *B*, spleen of a patient in whose clinical history the bowel movements were described as regular. The oleophages and larger collections are adjacent to the central arteries of the malpighian corpuscles. × 306.

addition to the 23 cases there were 7 instances in which sections of the liver showed vacuolated cells in or near the triads; it is possible that the mesenteric lymph nodes in these cases would have shown the lesions.

The clinical histories of the 23 patients in whose mesenteric lymph nodes the oleophages were found were studied; the correlation between the clinical histories and the microscopic changes is:

Clinical History	Oleophages Present
Liquid petrolatum used	5
Laxatives used for constipation	7
Use of laxatives denied	4
Clinical history not known	7

The history obtained in one of the cases of this group is given:

Case 1.—A man of 49 years was admitted to the hospital with the diagnosis of subacute bacterial endocarditis. He remained in the hospital until his death, a total of one hundred and eight days. In the "inquiry by systems" on admission, it was noted that the patient suffered from occasional constipation and that he took liquid petrolatum every morning. During the one hundred and eight days in the hospital he received a total of 1,530 cc. of liquid petrolatum and 1,335 cc. of liquid petrolatum emulsified with agar. The amount given the patient varied from day to day; up to 60 cc. of oil was given on some days, while on other days smaller amounts of oil or of oil emulsified with agar were given.

At autopsy the clinical diagnosis was confirmed. Microscopic examination of the color revealed in the basal portion of the lamina propria a few scattered vacuolated cells, the vacuoles unequal in size. No giant cells were seen. In the mesenteric lymph nodes were numerous vacuoles inside and outside cells. Most of the vacuoles were located either in peripheral lymphatic follicles or in medullary cords, but a few appeared to be in the central sinuses. About some of the larger extracellular vacuoles were clusters of smaller vacuoles. Six of the mesenteric lymph nodes were examined, and these oleophages were found in all, but the quantity varied in each lymph node, some sections showing many intracellular and extracellular vacuoles and others showing only one or two small vacuolated cells. In the liver many of the triads contained small collections of vacuolated cells similar to those seen in the colon, and similar groups were found focally in some of the lobules. These cells were surrounded by small lymphatic cells. The triads all contained an increased number of round cells. In many of the malpighian corpuscles of the spleen were collections of vacuolated cells similar in all respects to those in the liver. No extracellular vacuoles were seen.

Frozen sections of the colon stained with sudan IV revealed much more sudanophilic material in the lower portion of the mucosa than was indicated in the hematoxylin-eosin sections. This material failed to reduce osmic acid; when stained with the Carminati stain it was yellow, in sharp contrast to the orange color of the adipose tissue of the submucosa. The vacuolated areas in the mesenteric lymph nodes and in the hepatic triads contained material demonstrating the same reactions with osmic acid and orange sudan G as the material in the colon.

The appearance of the tissues in the other cases was essentially the same. However, in many of the lymph nodes numerous giant cells were

present, chiefly crescentic, surrounding the extracellular vacuoles, but occasionally appearing to lie free and resembling the Langhans type of cell.

In order to determine more definitely any frequency of absorption of liquid petrolatum in human subjects, the mesenteric lymph nodes, livers and spleens from 25 consecutive autopsies performed at Albert Merritt Billings Hospital on bodies over 21 years of age were examined. Since the previous work had indicated that lesions in the intestine would be present only in the event of marked changes in the mesenteric lymph nodes, microscopic examination of the intestines was made only in the cases in which oleophages were observed in the lymph nodes. These tissues were examined without knowledge of the use of laxatives during life; but subsequently the histories were studied.

The correlation between the patients' histories as regards the use or possible use of liquid petrolatum and the microscopic observations is:

Clinical History	Oleophages Present	Oleophages Not Found
Liquid petrolatum used	7	4
Laxatives used for constipation	4	2
Use of laxatives denied	1	4
Clinical history not known	3	

The history obtained in 1 of the cases of this group is as follows:

CASE 2.—A man of 54 years was admitted to the hospital with the diagnosis of carcinoma of the lung. In the "inquiry by systems" it was noted that the patient suffered from mild constipation. During the sixty-eight days that he was in the hospital he received a total of 1,440 cc. of liquid petrolatum.

Autopsy confirmed the clinical diagnosis. Microscopic examination of the intestines revealed no abnormalities. Scattered throughout two of the three mesenteric lymph nodes examined, lying in the peripheral lymphatic follicles and the medullary cords, were nests of vacuolated cells and large extracellular vacuoles similar to those previously described. In apposition to some of the larger vacuoles were crescentic giant cells with eosinophilic cytoplasm; in some of these giant cells vacuoles could be seen. In some of the follicles were nests of eosinophilic mononuclear cells showing no vacuolation. Frozen sections of the lymph nodes revealed the vacuolated areas to contain a sudanophilic material, which failed to reduce osmic acid and which stained yellow with the Carminati stain in contrast to the orange color of the surrounding adipose tissue. In the liver some of the triads contained cells with small vacuoles of varying size, surrounded by lymphocytes. Frozen sections revealed a sudanophilic material, part of which reduced osmic acid, while another part failed to reduce it. With the Carminati stain, no evaluation could be made of yellow-staining material in the triads because of lack of contrast with the surrounding liver cells and red blood cells. In several of the malpighian corpuscles of the spleen were small nests of vacuolated cells similar to those in the liver and lymph nodes. No extracellular vacuoles were seen. In frozen sections the sudanophilic material in these cells stained irregularly, some reducing osmic acid and other portions not reducing it, and some parts staining yellow and other parts orange with the Carminati stain.

In this study of human tissues there was found only partial correlation between the clinical histories and the microscopic changes; in about one third of the 25 consecutive cases the microscopic observations were directly opposite to what the clinical histories suggested. There are at least two possible factors in this lack of correlation. Many of the clinical histories are doubtless inadequate; many patients do not regard liquid petrolatum as a laxative, and unless specific inquiry is made its use will not be mentioned. A second factor is technical; it was found in this study that if several of the mesenteric and hepatic hilar lymph nodes from the same person were examined, macrophages containing oil might be present in some of the nodes while the remaining nodes appeared normal. Thus in some of the cases in which oil was not observed examination of other nodes might have permitted a different conclusion.

In 15 of the 25 consecutive autopsies, or 60 per cent, the lymph nodes revealed the presence of oil. This percentage might have been even greater if more nodes had been examined in some of the cases in which presence of oil was reported not found. In 6 cases, or 24 per cent, there were sufficient oleophages to warrant a + + or greater grading. In 4 livers, or 16 per cent, oil was demonstrable by microscopic examination. No oleophages were found in any of the sections of intestines. In 6 cases, or 24 per cent, there were vacuolated macrophages in the spleen. Pinkerton and Moragues [27] recently described the presence of similar cells in the spleen and liver in a case of paraffinoma of the lung; in the cases of pneumonia due to aspiration of liquid petrolatum observed in this laboratory the findings have been similar. Thus there are two possible sources for the splenic and hepatic lesions. In the cases in this series, however, the cells were present in the mesenteric lymph nodes whenever they were present in the liver and spleen, and no similar cells were seen in the lungs.

These findings in the human subject are similar to those in the experimental animal. The human subject first shows lesions in the mesenteric lymph nodes; lesions in the intestines are not frequent but may be present in cases of ingestion of liquid petrolatum over a long period. Giant cells are much more frequent and conspicuous in the human subject, but in other respects the location and morphologic character of the lesions are the same.

CHEMICAL ANALYSES

In an attempt to recover any liquid petrolatum that might be present in the various organs, some of the tissues were chemically analyzed. There were two main reasons why such a procedure seemed desirable. Although the appearance of the oleophages could be considered

27. Pinkerton, H., and Moragues, V.: Arch. Path. **29**:691, 1940.

characteristic of oil absorption, and the various staining methods gave supporting evidence concerning the nature of the contained material, it was felt that more conclusive proof would be available if a substance similar to liquid petrolatum could be recovered from the tissues. Chemical analyses would also provide information as to the extent to which absorption of this oil could occur quantitatively. This portion of the work was done under the direction of and with the assistance of D. Warren Stanger, of the Otho S. A. Sprague Memorial Institute.

The method used consisted of two parts: (1) the determination of the percentage of the unsaponifiable fraction of the test and of the control tissue and (2) the separation of the various components of the unsaponifiable fraction by chromatographic adsorption.[28] To determine the unsaponifiable residue the macerated tissue was saponified in alcoholic potash (a solution of potassium hydroxide in 95 per cent alcohol), extracted with ethylene dichloride and the solvent evaporated by vacuum distillation until the residue attained a constant weight. This residue was again saponified and extracted. The fractionation of the various components of an unsaponifiable mixture of materials soluble in fat solvents by chromatographic adsorption is based on the differing affinity possessed by activated aluminum oxide for the various components. In general, the more saturated the substance the less tendency will it have to be adsorbed to the particles of aluminum oxide, and compounds with certain active groups, such as the sterols, will have more tendency to be adsorbed than will the unsaturated hydrocarbons. In a mixture of cholesterol, carotene and liquid petrolatum there will be no adsorption of the saturated hydrocarbon and the carotene will be displaced by the cholesterol. Thus, if a mixture of the three fractions dissolved in purified petroleum benzine U. S. P. (petroleum ether) is poured into a column of activated aluminum oxide, the liquid petrolatum will be found in the lower portion of the column, the carotene in the center and the cholesterol nearest the top. Since the carotene is a colored substance, it will provide a line of demarcation between the other two fractions. When the column has been washed sufficiently to drive the carotene line toward the bottom, it will be known that all the liquid petrolatum is in the solvent in the flask below the column and that the cholesterol is attached to the aluminum oxide above.

The usefulness of this method was tested by a preliminary experiment in which weighed amounts of liquid petrolatum and cholesterol were dissolved in purified petroleum benzine, a minute amount of carotene added and the solution fractionated by use of the column of activated aluminum oxide. The results were as follows:

	Amount Used	Amount Recovered
Cholesterol	1.0027 Gm.	
Liquid petrolatum	0.0994 Gm.	0.0992 Gm.

Thus 99.8 per cent of the liquid petrolatum used was recovered.

Rabbit, rat, guinea pig and human tissues were analyzed by this method. The complete details of a typical analysis are:

Rabbit 29: A description of the feeding and the gross and microscopic observations on the tissues of this animal have been given. For the chemical analysis

28. Zechmeister, L., and von Cholnoky, L.: Die chromatographische Adsorptionsmethode, Berlin, Julius Springer, 1938.

of the liver, 92.8 Gm. of wet tissue was available. To this, after its maceration in a food chopper, was added 115 cc. of 6 per cent alcoholic potash, and the mixture was heated on the steam bath for eight hours. Then 60 cc. of distilled water was added, and the unsaponifiable material was extracted with 150 cc. of ethylene dichloride. The extraction was repeated three times, with 75, 75 and 50 cc. The water was then removed with anhydrous sodium sulfate, and the ethylene dichloride was evaporated by vacuum distillation until the residue had been brought to a constant weight. The weight of the unsaponifiable residue was 3.919 Gm. To this was added 15 cc. of 6 per cent alcoholic potash, the mixture heated as before and 20 cc. of distilled water added. Extraction was done with 25, 15, 15 and 15 cc. of ethylene dichloride. The water was again removed with sodium sulfate, and the ethylene dichloride was distilled from the residue. The weight of the unsaponifiable residue was then 2.9077 Gm. This material, which had an oily appearance, was dissolved in an excess of purified petroleum benzine, and to the solution a small amount of carotene was added. Not all of the residue was soluble, and only the supernatant fluid after centrifuging was poured through the column of activated aluminum oxide. After all of the solution had been added, the column was thoroughly washed by an excess of purified petroleum benzine. The solvent was evaporated by vacuum distillation until the residue attained a constant weight. The material thus recovered was clear and colorless and had the same appearance as the liquid petrolatum originally used. The amount recovered was 2.4517 Gm., which was 2.64 per cent of the wet weight of the liver.

The physical and chemical properties of this unadsorbed residue isolated from animals fed only one type of liquid petrolatum were determined and were compared with the corresponding properties of a fresh sample of the oil. The results were as follows:

	Index of Refraction at 23 C.	Specific Gravity at 30 C.
Unadsorbed residue.....	1.4690	0.8528
Light liquid petrolatum.....	1.4718	0.8587

The physical properties showed marked similarity. In the matter of specific gravity, the difference between that of the material isolated from the tissues and that of the fresh sample is only 0.0059; the range given by the manufacturers for the type of oil used is 0.0100. Thus the difference found here is about one half of that possible, according to the specifications of the oil. The oil used in feeding the animals was purchased at varying periods and was not all from the same lot, as was the fresh sample tested.

The chemical property determined was the absorption of hydrogen:

	Hydrogen Absorbed per Gram of Material, Standard Conditions
Unadsorbed residue.....	3.379 cc.
Light liquid petrolatum.....	1.052 cc.

A greater amount of hydrogen was absorbed by the final residue, but this included the hydrogen taken up by the material other than liquid petrolatum; the amount of hydrogen actually absorbed was very small, and this indicates that the product isolated from the tissues was practically saturated.

The technic of the analyses of the other tissues was similar to that described in all details. The results of the various analyses are given (all figures are the percentages of the wet weights of the tissues):

Rabbits.—Rabbit 29: This animal was fed liquid petrolatum for thirteen months. The details of the feeding and the results of the microscopic examination have been given. To make more certain that none of the material recovered was material adherent to the surface of the mucosa, the animal was fed the regular diet without the liquid petrolatum for three weeks before being killed.

Rabbit 33: This normal adult rabbit was used for control tissues.

	Unsaponifiable Residue (%)	Unadsorbed Residue (%)
Rabbit 29, intestine.....	2.85	2.24
Rabbit 33, intestine.....	0.45	0.02
Rabbit 29, liver.....	3.13	2.64
Rabbit 33, liver.....	0.45	0.04

Rats.—Rats 17 and 20: The tissues from these two animals were used together. Both animals had been fed on the bread and liquid petrolatum diet as previously described—rat 20 for six months and rat 17 for nineteen months. The feeding and the microscopic observations have been described.

Rats OC and YC: These are the control animals of the second group of rats, receiving no liquid petrolatum.

	Unsaponifiable Residue (%)	Unadsorbed Residue (%)
Rats 17 and 20, liver.....	8.60	6.37
Rats OC and YC, liver....	0.57	0.03

Rats OT and YT: The tissues from these animals were used together. These rats were members of the second group, which had been fed 5 per cent liquid petrolatum mixed with their diet for six weeks.

Rats OC and YC: The tissues from these animals were also used together. These were the control animals of the second group and received no liquid petrolatum.

	Unsaponifiable Residue (%)	Unadsorbed Residue (%)
Rats OT and YT, liver...	0.92	0.48
Rats OC and YC, liver...	0.57	0.03

Guinea Pigs.—Guinea pigs 2, 5, 18, 30, 36: Since only small portions of tissue fixed in solution of formaldehyde were available from the test animals, the tissues from the 5 guinea pigs were combined. These animals had been fed for periods varying from eight to fifteen months. The amount of oil given ranged from 800 to 1,200 cc.

Guinea pig C: The control animal was selected from a group which had been fed the stock laboratory diet.

	Unsaponifiable Residue (%)	Unadsorbed Residue (%)
Guinea pigs 2, 5, 18, 30, 36, liver...	0.43	0.054
Guinea pig C, liver.....	0.29	0.007

A close correlation exists between the microscopic observations and the results of the chemical analyses. In those tissues in which many

macrophages containing oil were demonstrable, the quantity of the final unadsorbed residue, probably chiefly oil, was from one hundred to two hundred times as great as the unadsorbed residue of the control tissues. The results of the experiment with the rats fed liquid petrolatum for only six weeks deserve special note. After this comparatively brief period on a diet containing 5 per cent of liquid petrolatum, the livers contained sixteen times as much residue as did the livers of the control rats. In the livers of the test rats no microscopic lesions were demonstrable; the oil was probably in too small quantities in individual macrophages to be recognized. That this may be true of many other tissues can only be suggested.

In the control tissues a very small quantity of residue not adsorbed to the aluminum oxide is present. The determination of the exact chemical nature of this material is beyond the scope of this investigation; its presence does not alter the conclusions.

Man.—In the interpretation of the results of the analysis of human tissues for ingested liquid petrolatum, no value can be given to the clinical histories regarding any use of the oil. The microscopic examination of human tissues showed that typical macrophages containing oil were present in the mesenteric lymph nodes and other tissues of patients who had not mentioned any use of laxatives. Thus it is difficult to obtain satisfactory control tissues. The use of the tissues of an infant dying before any liquid petrolatum is likely to have been given is not possible, because in the analyses of the tissues of the control rats it was found that there is an increase with age in the quantity of the unsaponifiable and unadsorbed residues. Despite this difficulty, analyses were made of the livers of two adults.

1. The details of this case have been given on page 686. There is a positive history of the use of liquid petrolatum, and the typical oleophages could be demonstrated microscopically in the liver.

2. The other patient was a girl of 22 years who died of chronic glomerulonephritis. The use of any laxative was denied; none was administered during the brief hospitalization, and no evidence of absorption could be seen on microscopic examination of the liver.

	Unsaponifiable Residue (%)	Unadsorbed Residue (%)
1. Liver	0.632	0.102
2. Liver	0.534	0.011

The demonstration of ten times as much unadsorbed residue in the test tissue as in the control seems to be significant. However, study of a larger series of cases would be necessary before definite conclusions could be drawn.

COMMENT

The results of these investigations demonstrate that liquid petrolatum is absorbed in small amounts from the intestines in human subjects and in experimental animals. This can be shown both by microscopic examination of the mesenteric lymph nodes and, in certain cases, of the

intestines, liver or spleen, and also by chemical analysis of the tissues. As a foreign body, the oil present in the tissues initiates a characteristic reaction. By morphology and staining methods, this lesion can be differentiated from the pathologic changes associated with the deposition of endogenous fats and lipoids. In the present work, as in that of Twort and Twort,[13] the ingested material was found in phagocytes. Tantini's[14] observations that the oil was stored in parenchymatous cells were based on the use of the Carminati stain. The lack of specificity of this stain as applied to hepatic tissue invalidates his conclusions. Both Twort and Twort and Tantini observed lesions only in the liver.

The results of the chemical analyses of the various tissues corroborate the conclusions of Channon and Collinson.[12] It is of additional interest that two different methods of analysis gave essentially the same results.

The method of absorption of the oil has not been demonstrated. Since liquid petrolatum is unsaponifiable, its absorption must be by a process other than that characteristic of ordinary fats and oils.

Pathophysiologic effects of the deposition of liquid petrolatum were not noted. The eventual reaction of the tissues to the presence of liquid petrolatum might vary. In pneumonia due to aspiration of liquid petrolatum and about paraffinomas in many locations there frequently occurs marked fibrosis of the surrounding tissues. Whether a comparable reaction occurs in the intestinal mucosa, the mesenteric lymph nodes, the liver or the spleen cannot be stated. The pathologic changes known to result from the presence of liquid petrolatum in the lumen of the intestine[29] were not observed in these experiments, probably because special precautions were taken to supply a diet rich in vitamins.

SUMMARY

Liquid petrolatum is absorbed in small amounts from the intestines of human subjects and experimental animals. The oil is demonstrable most readily in the mesenteric lymph nodes but may be present also in the intestinal mucosa, liver and spleen. A substance similar to liquid petrolatum in physical and chemical properties can be recovered from the unsaponifiable fraction of the involved tissues of animals fed this oil.

Liquid petrolatum is a foreign body in the tissues and excites a typical reaction, characterized by cells of chronic inflammation, including giant cells.

Pathophysiologic effects attributable to the deposition of liquid petrolatum in the tissues have not been demonstrated in this investigation.

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29. Jackson, R. W.: J. Nutrition **4**:171, 1931. Curtis, A. C., and Kline, E. M.: Arch. Int. Med. **63**:54, 1939. Elliott, M. C.; Isaacs, B., and Ivy, A. C.: Proc. Soc. Exper. Biol. & Med. **43**:240, 1940.

THE SYNOVIAL MEMBRANE IN CHARCOT'S JOINT

WITH SPECIAL REFERENCE TO THE GOLGI APPARATUS AND THE SYNOVIAL FLUID

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In disease of a joint, all the structures which take part in the joint are to some extent affected. Most of the descriptions of diseases of joints include mainly the more gross and obvious alterations in bone, cartilage, ligaments and other tissues and give little account of the synovial membrane. This paucity of information is specially apparent in the case of Charcot's disease of joints and since in this condition certain changes are more striking than in many other diseases of joints, they are described here.

In order to demonstrate the finer changes in cells, special attention has been given to the protoplasmic "organoids," especially the Golgi apparatus. Such investigations are of interest to surgeons because material must be fixed in suitable fluids immediately after removal from the body if satisfactory observations are to be made, and this can be done only if material is obtained at operation.

CHANGES IN SYNOVIAL MEMBRANE

The usual description is that of various retrogressive alterations, such as atrophy, degeneration or necrosis. There is no question whatever that in the joint cavity there is a considerable amount of necrotic and fibrinous material in juxtaposition to or lying in the synovial membrane. The surface of the membrane is often rough and shaggy. Closer observation shows that the membrane is often, if not invariably, thickened and swollen.

Microscopic examination shows necrotic material either lying free in the cavity or occurring in the synovial membrane and the villi. In addition to this material, masses of fibrin are to be seen on the surface or incorporated in the tissue.

In contradistinction to this there is, as in the less grotesque forms of osteoarthritis, considerable hyperplasia of the synovial cells, with an increase in the thickness of the synovial layer. The thickening and

From the University of Melbourne.

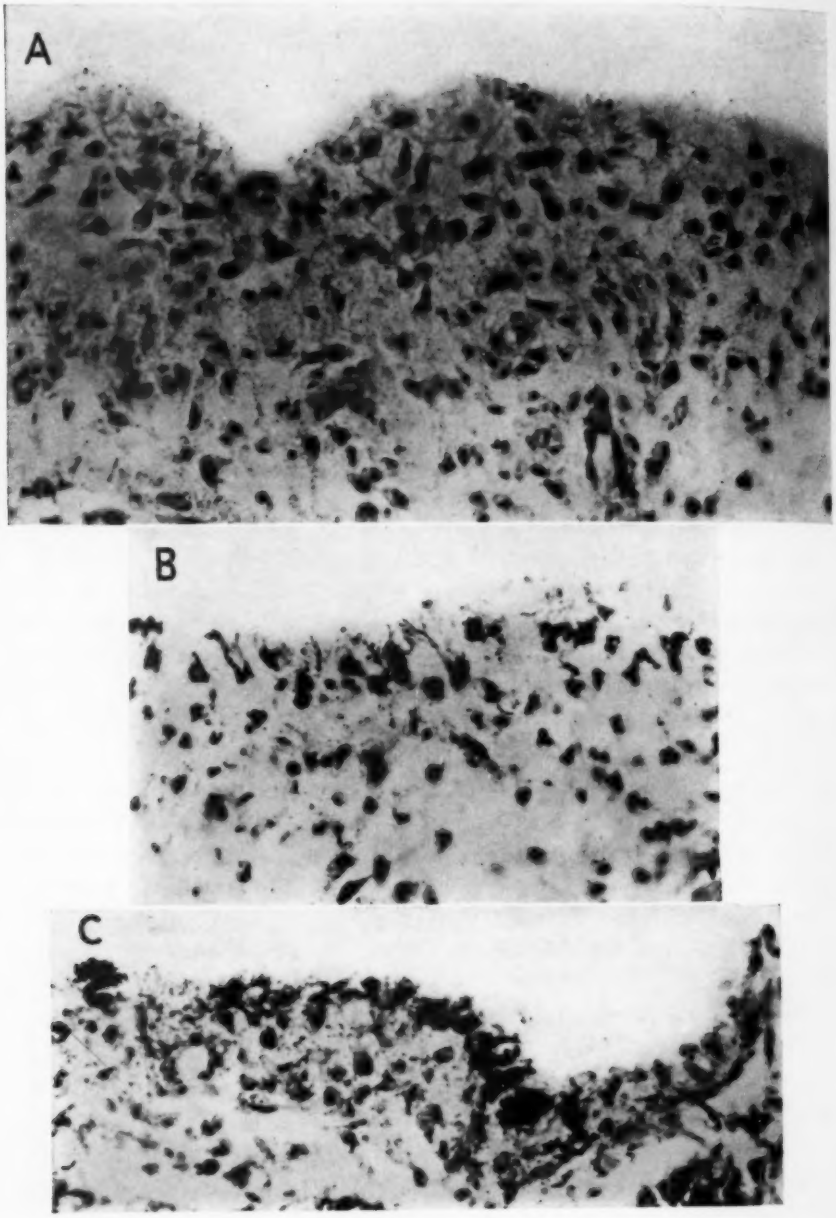

Figure 1

(See legend on opposite page)

overgrowth of the membrane are often associated with the formation of numerous and large villous projections.

Although both retrogressive and proliferative changes are found together, pieces of the lining membrane removed from almost any part of the joint usually show more proliferative than degenerative change.

HISTOLOGIC INVESTIGATION

The normal lining of the joint consists of the synovial layer and subjacent areolar fibrous or fatty connective tissue. The synovial layer consists of a connective tissue containing collagenous and elastic fibers, reticular fibers and a peculiar ground work which enables this layer to be distinguished very easily from the ordinary connective tissue beneath. The cells may be only one layer thick or there may be two or three layers. These cells lie in the connective tissue stroma–matrix, and some of them may lie on the surface; thus the surface may be lined by cells or may be composed of the connective tissue stroma itself.

In Charcot's joint, which may be regarded as presenting an exaggerated form of osteoarthritis, the membrane is usually considerably thickened and the cells are much more numerous than usual (fig. 1 *A*). An examination of a large number of specimens and of serial sections was made to determine that thickening and increase in cellularity are a true interpretation and not due to oblique or tangential cutting of a single-layered area. The synovial cells are enlarged and have well developed nuclei (sometimes more than one in a cell), and there is a well developed Golgi apparatus in the protoplasm.

In some places the synovial layer is relatively normal; i. e., the surface is well defined, the layer being thus demarcated from the joint fluid, and similarly the deeper surface is distinguishable readily from the subjacent tissue (figs. 1 *C* and 3 *B*). Collagenous fibrils are increased in number. In other areas, however, the demarcation of the membrane from adjacent material may be very poor; thus the superficial margin

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EXPLANATION OF FIGURE 1

A, photomicrograph of a portion of synovial membrane in a case of Charcot's disease of the knee joint, showing thickening of the membrane and hyperplasia of the synovial cells. The surface is ill defined, and the tissue appears to merge into the joint fluid. The Golgi apparatus of the cells are well developed; the lightly stained nuclei are apparent in some of the cells. All the specimens were impregnated by Da Fano's (cobalt nitrate–silver) method and counterstained with carmine.

B, photomicrograph of synovial membrane of a knee joint showing well developed synovial cells, some of which are merging into and passing into the joint fluid; × 323.

C, photomicrograph showing a palisade arrangement of some of the superficial cells; × 323.

is indefinite, the membrane—both intercellular tissue and cells—appearing to merge into the joint fluid (figs. 1 *B* and 3 *C*). On the deeper aspect the change from synovial tissue to ordinary connective tissue is very gradual, and it is difficult, if not impossible, to say where one begins and the other ends (figs. 1 *A* and 3 *C*).

The synovial cells adopt very different arrangements from any seen in the normal membrane. They are often closely packed together and show great variation in shape and size. On the surface they often

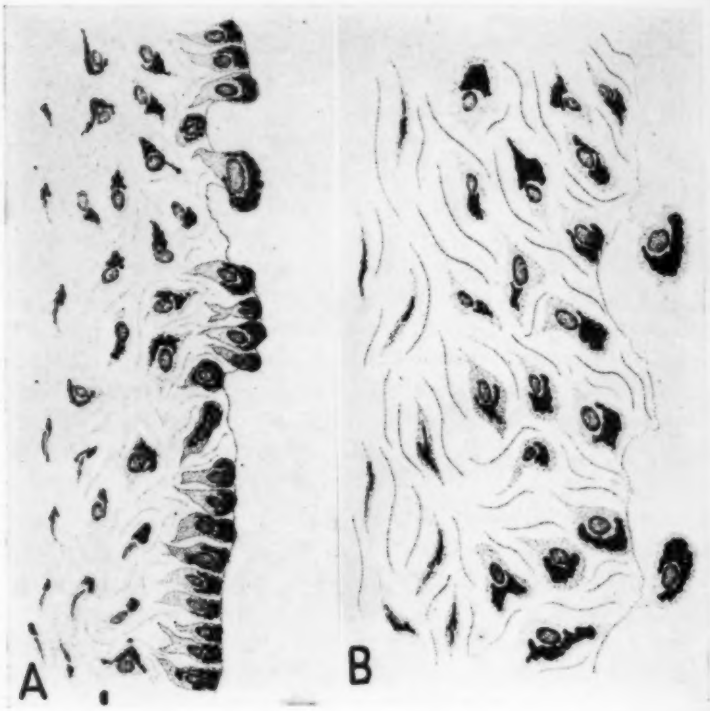
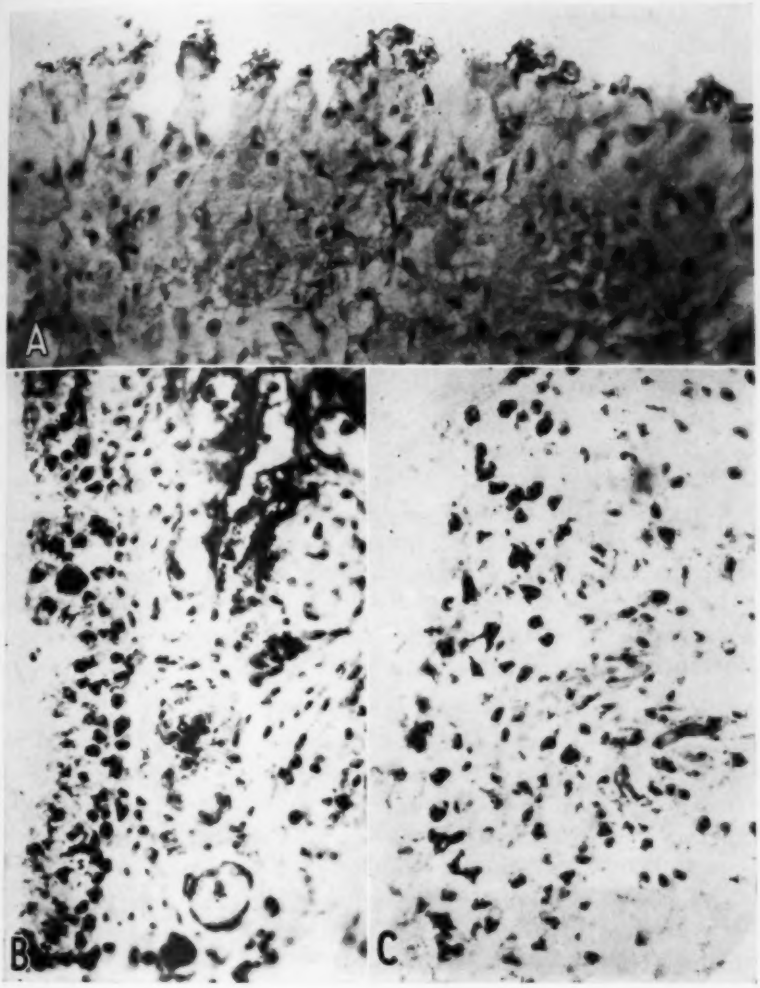


Fig. 2.—*A*, drawing of an area of synovial membrane to show the relationship of the Golgi apparatus to the nucleus and the protoplasm of the cell. Note the palisade arrangement of superficial cells and the beginning migration of some into the joint cavity. *B*, drawing of a portion of synovial membrane showing an irregular surface and passage of cells into the joint cavity.

become orientated so that their long axis is at right angles to the margin of the membrane, and in this way they give a palisade appearance, which at first glance may closely resemble columnar epithelium (figs. 1 *C* and 2 *A*). In other places the cells are so closely packed together that there is a superficial resemblance to pseudostratified epithelium (figs. 3 *B* and 4 *A*). In many areas, cells may be observed projecting from the surface

of the membrane (fig. 3 *A*). All stages may be seen from those in which the cell merely projects somewhat above the surrounding tissue, through those in which projection is exaggerated, to others in which the cell is becoming quite separate from the membrane. In some of these it can be



EXPLANATION OF FIGURE 3

A, photomicrograph showing a very irregular synovial surface, the cells apparently merging into the joint fluid; × 323.

B, photomicrograph of an area in which cellular proliferation gives an appearance not unlike pseudostratified epithelium; × 323.

C, photomicrograph showing hyperplastic membrane in which the cells are well defined and yet the surface is poorly demarcated from the joint fluid; × 323.

seen that part of the intercellular substance is being "drawn out" with the cell (fig. 2 *A*). It is appreciated that some of these appearances could be due to oblique sectioning of tissue, especially as folding of synovial membrane is common; but, as stated, serial sections have shown that the appearances cannot be explained as artefacts.

The general character of the Golgi apparatus of the normal synovial cells need not be discussed here, as it has been described elsewhere.[1] An arresting observation is this—that the Golgi apparatus should be so well developed in almost all the cells; in many of them it is greatly enlarged and occupies a considerable part of the protoplasm. Thus the desquamating and desquamated cells show this structure in a very well developed form (figs. 2 *A* and *B* and 3 *A*). Synovial cells lying free in the fluid and distinct from the membrane also show this structure.

In many cases of Charcot's disease there is an increase in the amount of synovial membrane in the joint. This is due to two factors: transudation from the blood vessels (and thus it is comparable with edema of other parts of the body) and an actual increase in the mucinous material —this being associated with an increase in the number of cells. Though these two factors are frequently associated, it is important that they should be distinguished.

The free synovial cells in the joint fluid may be demonstrated in material removed by aspiration. In fixed material (in sections) they are to be found in crypts and between folds of tissue, where they have been retained during the preparation of the material.

The increase in the fluid of the joint, especially when there is an increase in the cell content, is usually most obvious where synovial proliferation is most obvious.

COMMENT

The forms of activity and hyperplasia of synovial membrane have been described to indicate that progressive and proliferative changes in the membrane may be as obvious and important as any atrophic or degenerative processes. There is, however, another feature worthy of attention. As pointed out in a previous paper,[1] observations in this type of material may support the hypothesis of the tissue nature of synovial fluid.

In 1930 H. T. Jones [2] suggested that abnormal bursae were formed by liquefaction of intercellular connective tissue. In 1933 Vaubel [3] proposed the hypothesis to apply to all synovial fluids, both those in normal joints and those in abnormal ones.

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1. King, E. S. J.: J. Path. & Bact. **41**:117, 1935.
2. Jones, H. T.: J. Bone & Joint Surg. **12**:45, 1930.
3. Vaubel, E.: Virchows Arch. f. path. Anat. **289**:670, 1933.

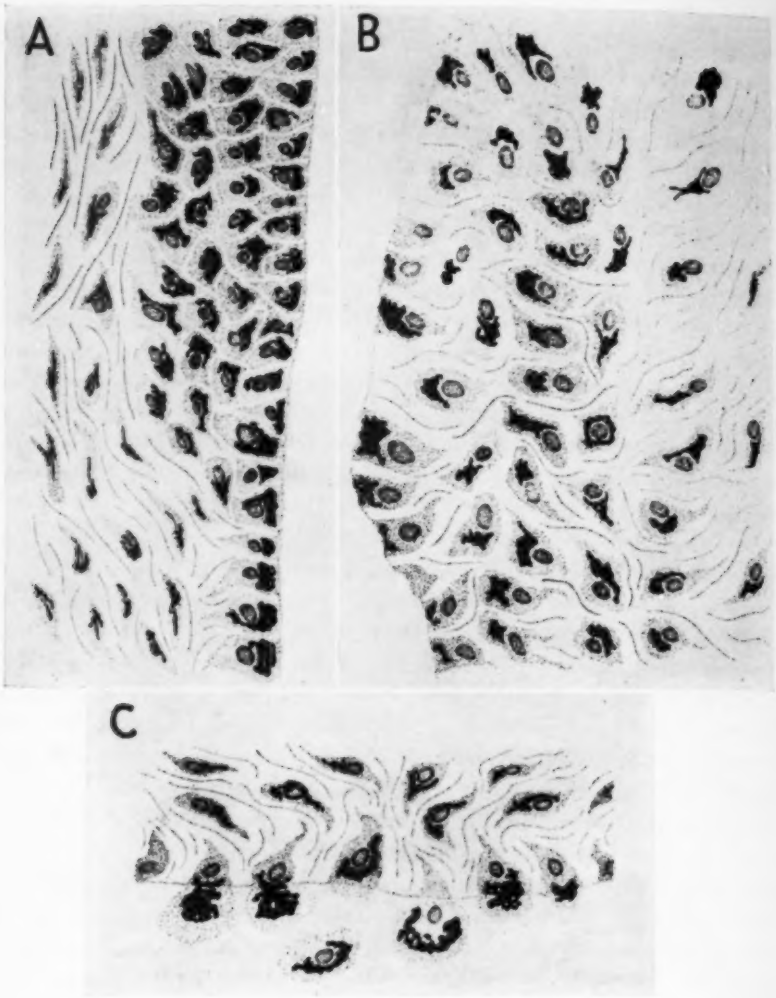
A number of the observations made in these active tissues are specially significant: 1. The poor demarcation of the membrane and the cells from the adjacent synovial fluid has been described in an earlier paragraph. 2. Cells may be observed passing from the membrane into the fluid. 3. Parts of cells may be seen in the membrane, and the appearances strongly suggest that the other parts of these have become separated (because of their more fluid character) during the preparation of the section (fig. 4 *B*). 4. Cells which are becoming separated or which are lying in the synovial fluid are seen to be quite healthy as judged by their well formed Golgi apparatus (fig. 2 *B*).

The problem of the form of the Golgi apparatus during degeneration has been studied both by late fixation of tissue and in tissue which contains areas of retrogressive change recognizable by ordinary observations. The Golgi apparatus becomes granular and disappears (i. e., is not demonstrable) in the cell at a stage at which there is no significant change in the nucleus or the protoplasm. This question is to be discussed elsewhere.

The poor demarcation of the synovial layer from the subjacent connective tissue may be due to the projection of synovial cells into this, as a mechanical effect of the hyperplasia. This hyperplasia undoubtedly occurs in the synovial layer, as it does also, to some extent, in the connective tissue cells. It is probable, however, that proliferating connective tissue cells, which become swollen and have a more voluminous protoplasm, have changed in type and become synovial in character, and are added to the synovial layer. The morphologic appearances suggest that this is so, and incidentally experiments performed on the regeneration of synovial membrane from connective tissue indicate that such metaplasia is probably a common regional transformation. There is thus a gradual transformation in the tissues from fibrous or areolar connective tissue to synovial fluid: the connective tissue merges into the synovial layer and this into the synovial fluid.

The synovial fluid contains synovial cells (ignoring for the moment the various tissue or blood wandering cells which are common to all connective tissues) which are histologically normal. This fluid thus differs from adjacent connective tissues only in that the intercellular material is physically different.

Whether synovial fluid is regarded as a tissue continuous with, but more fluid than, the synovial membrane or as a secretion derived from this membrane depends partly on the significance attributed to the synovial cells in the fluid and on the relationship of the fluid to the connective stroma of the membrane. The two ideas are similar but not identical. The term "secretion" usually applies to material from, for example, the mucous membrane of the alimentary canal, which becomes



EXPLANATION OF FIGURE 4

A, drawing to show the arrangement of cells in an area where a "pseudostratified" area merges into a "columnar" or palisade zone.

B, drawing to show the general structure where the surface is poorly defined from the joint fluid. Some of the cells appear to have been partly removed (compare *C*). The appearances suggest that the peripheral part of the cells (that actually in the joint fluid) is more fluid and has been removed during the preparation of the section.

C, drawing to explain the appearance shown in *B*. Though this drawing is hypothetic, it is supported by observations such as those illustrated in figure 3 *A* and by others which are more exaggerated than this. The appearances illustrate the relationship between joint fluid and the synovial membrane in some areas and support the view of the tissue character of the fluid.

quite independent of, and is removed from, the neighborhood of the originating cells. On the other hand, in a tissue there are cells which lie in and control the stroma.

In the case of synovial fluid one has a material which may change very rapidly in amount; this may be either increased or diminished quickly. Although in part this is due to mere alteration in the amount of fluid and is comparable with the appearance and disappearance of fluid in other tissues, there are in addition alterations in the mucin and cell content.

The synovial fluid thus is regarded as a fluid tissue rather than as a secretion of the lining cells of the synovial membrane for the reasons that:

1. It contains cells which are morphologically (and incidentally there are many observations, which need not be discussed here, that show that they are also functionally) normal.
2. Changes occur in the fluid which are most readily explained on the assumption that the fluid is under definite cellular control.
3. In abnormal conditions, especially if the tissues are very active, the synovial membrane may be shown to merge into the fluid.

Synovial fluid thus may be regarded as a connective tissue with a very fluid matrix, and from the point of view of the physical character of intercellular matrix, the various connective tissues exhibit a gradation from bone at one extreme, through cartilage and the various forms of fibrous connective tissue, mucoid tissue, to synovial fluid at the other.

SUMMARY

In Charcot's joints, in addition to retrogressive changes there are well developed and easily recognizable progressive and proliferative changes in the synovial membrane. The relationship between synovial membrane and fluid may be observed readily in this condition. A consideration of this relationship and of the histologic character of the cells in the synovial fluid indicates that the hypothesis that this fluid is an actual connective tissue—only differing from other connective tissues in that the intercellular matrix is fluid—is the most satisfactory explanation of the observations.

SPONTANEOUS FIBROMYOMA IN THE FEMALE GUINEA PIG

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SANTIAGO, CHILE

Uterine fibroids can be induced in the guinea pig by prolonged treatment with estrogens (Nelson [1]; Lipschütz and Iglesias [2]; Iglesias [3]; Moricard and Cauchoix [4]). The question arises whether uterine fibroids may occur in the guinea pig spontaneously.

Literature on spontaneous tumors in the guinea pig is very scarce (Heim and Schwartz [5]). The only tumors which occur spontaneously in this species with a certain frequency seem to be the following: an adenoma-like neoplasm of the lung; chorioncarcinoma of the ovary, discovered by Loeb [6] (1911) and recently described in much detail by Athias [7]; adenoma and lipoma of the mammary gland. Some other neoplasms, among them a subcutaneous sarcoma and a sarcoma of the myometrium, have been described (quoted from Heim and Schwartz [5]). No data are as yet available in the literature as to fibroid or fibromyoma, uterine or extrauterine, in the guinea pig.

In this paper are described several observations of spontaneous uterine tumors in the guinea pig, and the question of the frequency of spontaneous uterine or extrauterine fibroids in the same species is discussed.

REPORT OF CASES

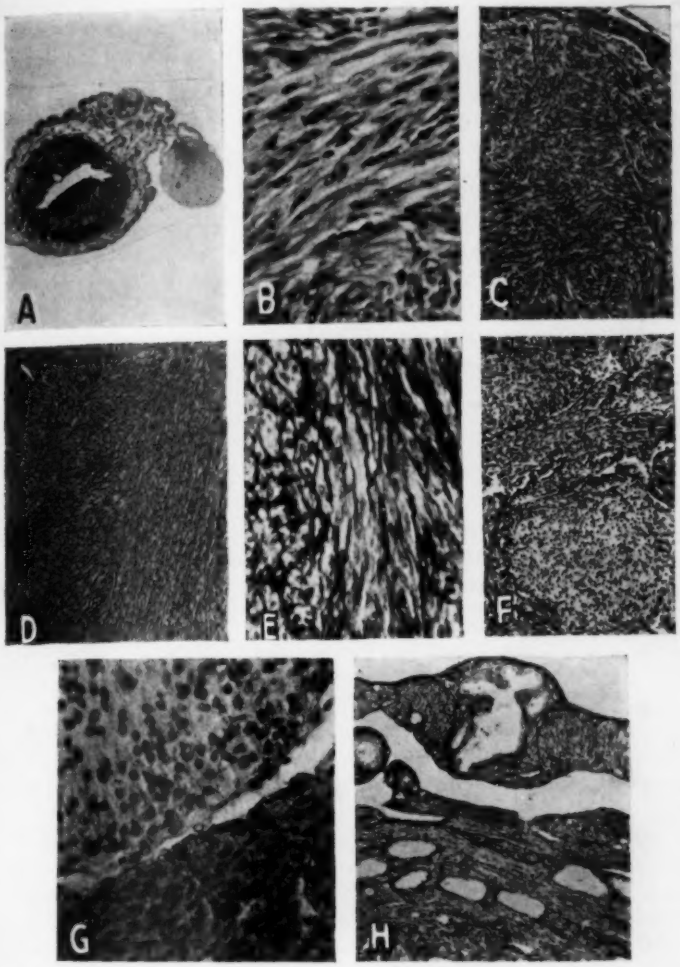
CASE 1.—A small pedunculated mesometrial nodule of a diameter of about 2 mm. (*A*, *B* and *C* in figure) was found in a female belonging to a lot of aged animals

From the Department of Experimental Medicine, National Health Service of the Republic of Chile.

This investigation has been aided by a grant from the Ella Sachs Plotz Foundation for the Advancement of Scientific Investigation and from The Jane Coffin Childs Memorial Fund for Medical Research.

1. Nelson, W. O.: Anat. Rec. **68**:99, 1937; Endocrinology **24**:50, 1939.
2. Lipschütz, A., and Iglesias, R.: Compt. rend. Soc. de biol. **129**:519, 1938.
3. Iglesias, R.: Tumores experimentales uterinos y extragenitales provocados por el benzoato de estradiol, Publication of the Department of Experimental Medicine, National Health Service of the Republic of Chile, Chile, 1938.
4. Moricard, R., and Cauchoix, I.: Compt. rend. Soc. de biol. **129**:556, 1938.
5. Heim, F., and Schwartz, P.: Die Spontantumoren der Meerschweinchen, in Jaffé, R.: Anatomie und Pathologie der Spontanerkrankungen der kleinen Laboratoriumstiere, Berlin, Julius Springer, 1931, pp. 734-739.
6. Loeb, L.: J. A. M. A. **56**:1327, 1911; cited by Athias.[7]
7. Athias, M.: Arq. de pat. **8**:122, 1936.

killed in order that the condition of the normal uterus might be studied. The tumor was very hard. Microscopically it was a fibromyoma. Its structure was similar to that of fibromyoma in woman (*D* and *E* in figure). On the other hand,



A, B, C, from a spontaneous uterine fibroid found in an untreated guinea pig; *A,* × 5; *B,* × 200; *C,* × 45. Note capsule and orderly disposition of muscle tissue. Hematoxylin and eosin.

D, E, from a small subserous fibroid of the body of the uterus of a woman 22 years old; *D,* × 45; *E,* × 200. Van Gieson's stain.

F, G, from small intramural nodules in the uterus of an untreated guinea pig; *F,* × 45; *G,* × 200. The nodules consist of large cells with clear protoplasm, probably hyperplastic muscle fibers. Van Gieson's stain.

H (× 45), subserous cyst is a guinea pig treated with free estrone for ninety-eight days (see group 3 in table). Van Gieson's stain.

the structure was different from that of experimental fibroids (Lipschütz, Vargas and Iglesias [8]; Lipschütz and Vargas [9b]).

Case 2.—Small intramural nodules consisting of large cells, probably hyperplastic muscle fibers, were discovered in the myometrium of an aged untreated female guinea pig (*F* and *G* in figure). There was no capsule separating the nodule from the surrounding muscle tissue, though the limiting line between both was a rather sharp one. The diameter of the bigger nodule was less than 1 mm.

Case 3.—A subserous tumor, just visible to the naked eye, having a diameter of about 0.5 mm., was discovered in a castrated female which received during fourteen weeks 5 micrograms of estrone (theelin) thrice weekly by subcutaneous injection. The nodule was thought, at first sight, to be a fibroid. Such a finding was not expected, since quantities about thirty times greater are necessary to produce fibroids in the guinea pig by a prolonged treatment with free estrogens (Lipschütz and Vargas [9a]; Lipschütz, Rodriguez and Vargas [10]; Rodríguez [11]).

Development of Tumors in a Group of Female Guinea Pigs Untreated, Treated with Vegetable Oil or with Nontumorigenic Doses of Estrogens

Group	Description of Animals	Animals	Number with Tumors
1	Noncastrated, untreated adults, virginal or old, which served for breeding; bought from different dealers	94	1 with small pedunculated fibroid of the uterus 1 with intramural myomatous nodules
2	Noncastrated, treated with 0.4 cc. of olive oil thrice weekly during almost 5 months	4	0
3	Castrated and then treated with 1 to 80 micrograms of free estradiol and estrone thrice weekly during 3 months	60	1 with subserous cystic nodule
4	Castrated and then treated with 0.1 microgram of different esters of estradiol (monobenzoate, dipropionate, benzoate-butyrate, octoate) thrice weekly during 3 to 3½ months	20	0

Microscopic examination revealed that the small tumor was not a fibroid (figure, *H*) but a cystic structure lined by cuboidal epithelium. A similar cystic structure was found at another place beneath the serosa; an accumulation of concentric smooth muscle fibers was present between serosa and cyst at this place. It is impossible to say whether these structures were enlarged uterine glands which penetrated into the muscular coat and reached the serosa, or a rest of the wolffian duct. The first would be a rather unexpected phenomenon, since the uterus had in this case a normal weight (1.6 Gm.) and an almost normal microscopic aspect.

8. Lipschütz, A.; Vargas, L., Jr., and Iglesias, R.: Compt. rend. Soc. de biol. **129**:524, 1938.

9. Lipschütz, A., and Vargas, L., Jr.: (*a*) Compt. rend. Soc. de biol. **130**: 9, 1939; (*b*) Lancet **1**:1313, 1939.

10. Lipschütz, A.; Rodríguez, R., and Vargas, L., Jr.: Compt. rend. Soc. de biol. **130**:939, 1939.

11. Rodríguez, F.: Estudio experimental comparativo sobre la dosis histerotrófica útil y la dosis tumorígena de las hormonas foliculares libres (estradiol y estrona), Publication of the Department of Experimental Medicine, National Health Service of the Republic of Chile, Chile, 1940.

The only spontaneous uterine fibromyoma described (case 1) was found in a group of 35 adult untreated animals. Later on, a careful search for uterine tumors visible to the naked eye was made by my assistant Dr. Vargas in 59 adult untreated animals. Not another one was found. This gives full evidence that it is extremely rare to find spontaneous uterine fibroids in untreated animals.

The third specimen described, in which there was a small subserous cystic nodule, was found in a group of 60 castrated animals which received for three months thrice weekly subcutaneous injections of 1 to 80 micrograms of free estradiol (dihydrotheelin) or estrone. These quantities are sufficient to maintain normal uterine weight and structure or to produce considerable uterine hypertrophy. In the table I have added other groups of animals treated with quantities of estrogens (esterified estradiol) sufficient to promote normal uterine development; there was among these 80 females (groups 3 and 4) no animal with a subnormal uterine weight. No uterine fibroids were found. No extrauterine tumors were found in the 178 animals of the four groups.

From my experience of former years with guinea pigs I remember only 1 animal which died untreated and which at necropsy had an abdominal tumor attached to the bowels. The diameter was about 3 to 4 cm. The pathologist declared it to be, according to the microscopic structure, a "fibroid." An abdominal tumor of the lumbar region, the size of a pigeon's egg, has been described recently by Kröning and Wepler [12]; it was a fibroma with lipomyxomatous zones.

SUMMARY

Spontaneous uterine or extrauterine fibroid and fibromyoma are extremely rare in the guinea pig. One small pedunculated uterine fibromyoma was found in a group of 94 untreated, noncastrated adult females. Its microscopic structure was similar to that of fibromyoma in woman and different from that of experimental fibroid in the guinea pig. In another untreated female intramural nodules of hyperplastic muscle fibers were discovered in the uterus during microscopic examination.

12. Kröning, F., and Wepler, W.: Ztschr. f. Krebsforsch. **48**:256, 1938; abstracted, Index analyt. de cancérol. **13**:354, 1939.

RELATION OF VITAMIN E TO THE EFFECTIVENESS OF TESTOSTERONE INJECTED INTO CAPONIZED MALE FOWLS

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URBANA, ILL.

Since vitamin E is so closely associated with the reproductive system in vertebrates, the question arises as to whether or not it has any role to play in the utilization of the hormones affecting this system. That it does is suggested by the fact that vitamin E deficiency produces testicular degeneration in the male rat and that this condition is followed by the appearance of so-called castration cells in the hypophysis (Van Wagenen [1]; Nelson [2]). The interstitial tissue appears to be unaffected, however, and the secondary sex characters and accessory sex glands are maintained in almost the normal state, indicating continued production of hormone. Kudrjaschov [3] described progressive atrophy of the prostate and seminal vesicles in the male rat, followed by eventual loss of interest in the female. This, according to Mason,[4] may be attributed to poor physical condition and retarded growth. Weisener and Bacharach [5] also noted a change in sex behavior similar to that following hypophysectomy, which, however, was not cured by vitamin E supplements.

The loss of sex interest in the male fowl appears before any marked degeneration of the testis and before the secondary sex characters show regression (Adamstone [6]). This also indicates a relation between vitamin E and the utilization of androgen. In investigating this possibility the fowl should be a very suitable experimental animal because of its prominent secondary sex characters. Accordingly, an experiment was carried out to compare the effectiveness of testosterone propionate injected into caponized vitamin E–deficient male fowl, some of which received vitamin E, while others did not.

From the Department of Zoology, University of Illinois.

1. Van Wagenen, G.: Anat. Rec. **29**:398, 1925.

2. Nelson, W. O.: Anat. Rec. **56**:241, 1933.

3. Kudrjaschov, B. A.: Endokrinologie **7**:91, 1930; Tr. Dynamics Development **6**:29, 1931.

4. Mason, K. E., cited by Allen, Danforth and Doisy,[8] p. 1149.

5. Weisener, B. P., and Bacharach, A. L.: Nature, London **140**:972, 1937.

6. Adamstone, F. B., and Card, L. E.: J. Morphol. **56**:339, 1934.

METHOD

Ten male white Leghorn chicks which had been fed an iron-treated, vitamin E-deficient ration[7] from the time of hatching were caponized when they reached the age of 67 days. All were in good condition, having escaped nutritional encephalomalacia and other reactions to vitamin E deficiency. They were maintained on the vitamin E-deficient diet until the end of the experiment. A control group of 4 white Leghorn males of the same stock were caponized and reared on the normal, untreated ration. As had been anticipated, several of the birds were imperfectly caponized, owing to the age at which the operation was performed, and one had a slipped tendon. These birds were eliminated from the experiment. The remainder showed the typical effects of caponizing, namely, regression of comb and wattles and assumption of a "lanky" appearance of the body (Allen[8]). The birds were divided into three groups, as follows:

Group A: Control; to receive injections of testosterone propionate.
Diet: untreated ration 152.

Group B: Experimental; to receive injections of testosterone propionate.
Diet: iron-treated, vitamin E-deficient ration.

Group C: Experimental; to receive injections of testosterone propionate,[9] plus doses of alpha tocopherol,[9] a synthetic vitamin E.
Diet: iron-treated, vitamin E-deficient ration.

Since the amount of testosterone propionate to be injected, as well as the size of the dose of alpha tocopherol, was a matter of conjecture under the conditions of the experiment, the question was settled somewhat arbitrarily. The birds were weighed, and since it was found that most of them were fairly close to 2,000 Gm., it was decided to give intramuscular injections of 2 mg. of testosterone propionate (dissolved in 0.2 cc. sesame oil) per bird and to administer by mouth 5 mg. doses of alpha tocopherol (dissolved in the vitamin A-D supplement) to each of the birds in group C.

EXPERIMENTAL RESULTS

The results of the experiment were well defined, as will be seen from the table and the illustration.

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7. The ration (no. 152) used in this experiment was composed of the following ingredients: ground yellow corn 46 parts, wheat bran 13, wheat middlings 13, alfalfa meal 5, meat scrap 10, dried skim milk 10, salt 1. As the vitamin A-D supplement, 0.5 per cent Nopco was used. Vitamin E in the ration was destroyed by treatment with a 1 per cent solution of ferric chloride in ether and subsequent evaporation of the ether (Waddell and Steenbock, 1928). Nopco is the trade name of a blend of vitamins A and D with cod and other fish liver oils, sold by the National Oil Products Company, Harrison, N. J.

8. Allen, E.; Danforth, C. H., and Doisy, E. A.: Sex and Internal Secretions, Baltimore, Williams & Wilkins Company, 1939.

9. The testosterone propionate used in this experiment was donated by the Schering Corporation, Bloomfield, N. J. The synthetic vitamin E or alpha tocopherol, was supplied by Merck & Co., Inc., Rahway, N. J.

All birds in group A, on the normal ration, responded immediately to the injected testosterone propionate. After the second injection their combs and wattles were greatly enlarged, firm, erect and bright red, like those of a normal male.

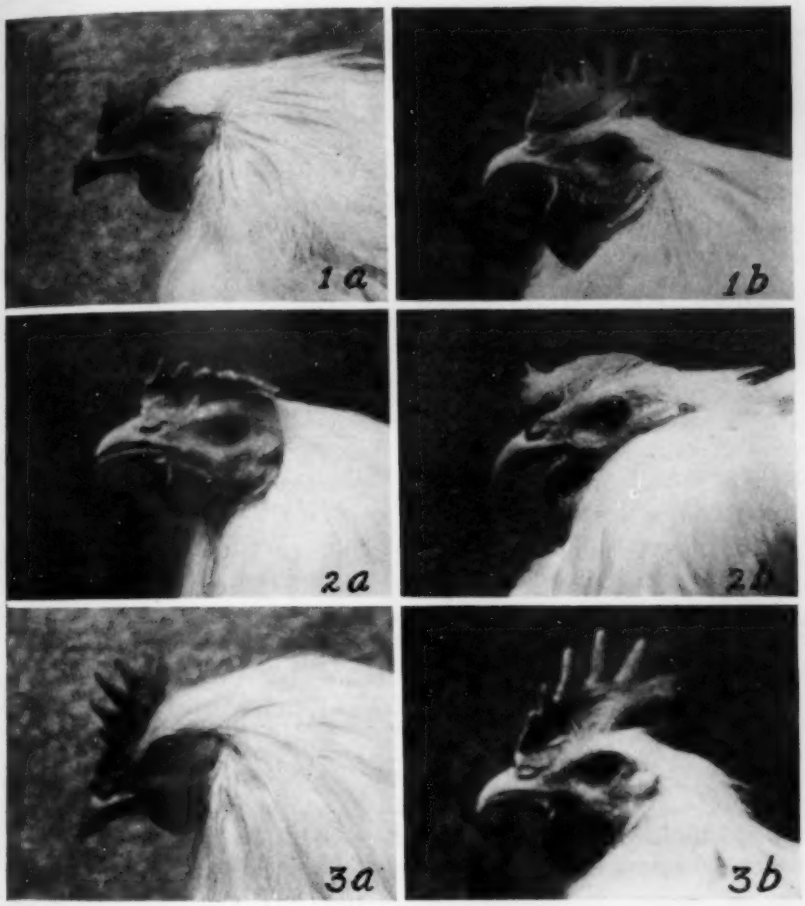
In group B, birds 1 and 2 responded fairly well to the injected substance but much more slowly than the controls. After ten days the head

Experimental Data

Group	Bird	Weight, Gm.	Condition of Comb and Wattles on Date of Injection of Testosterone Propionate							
			May 13	May 15	May 17	May 20	May 28	May 30	June 6	June 8
A	1	1,740	Shrunken, flaccid, pale	Larger, firmer, pink	Enlarged, erect, bright red	Rapid return to normal (4 days)				
	2	1,825	Shrunken, flaccid, pale	Larger, firmer, pink	Enlarged, erect, bright red					
	3	2,178	Shrunken, flaccid, pale	Larger, firmer, pink	Enlarged, erect, bright red					
B	1	1,735	Shrunken, flaccid, pale	Larger, droopy, pink	Larger, firmer, red	Slow return to near normal (10 days)				
	2	2,085	Shrunken, flaccid, pale	Larger, droopy, pink	Larger, firmer, red	Slow improvement (10 days)				
	3	1,995	Shrunken, flaccid, pale	Larger, droopy, pink	Larger, droopy, pink	Head furnishings improved in size and color but remained droopy				
	4	1,975	Shrunken, flaccid, pale	Larger, droopy, pink	Larger, droopy, pink					
C	1	1,545	Shrunken, flaccid, pale	Larger, firmer, red	Enlarged, erect, bright red	Rapid return to normal (4 days)				
	2	1,665	Shrunken, flaccid, pale	Larger, firmer, red	Enlarged, erect, bright red					
	3	1,950	Shrunken, flaccid, pale	Larger, firmer, red	Enlarged, erect, bright red					
	4	1,905	Shrunken, flaccid, pale	Larger, firmer, red	Enlarged, erect, bright red					

furnishings of bird 1 compared favorably with those of birds in group A, while those of bird 2 lagged considerably and the comb was still somewhat flaccid. Birds 3 and 4, however, gave little evidence of response, and the injections were repeated at intervals until a total of seven had been given over a period of twenty-four days. Even then the head furnishings never became erect like those of the normal group, although some improvement was noticeable in size and color.

The birds in group C showed an immediate and rapid response to the treatment after the first oral dose of alpha tocopherol and the first injection of testosterone propionate had been given. The combs and wattles became larger, strongly erect and brilliant red. The birds also became very active and aggressive.



1. Capon reared on normal untreated ration: (*a*) Regression of comb and wattles sixty-three days after caponizing. (*b*) Improvement in comb and wattles one week after the first injection of testosterone propionate.

2. Capon reared on iron-treated, vitamin E–deficient ration: (*a*) Appearance sixty-three days after caponizing. (*b*) Little improvement after seven injections of testosterone over a period of twenty-four days.

3. Capon reared on vitamin E–deficient ration supplemented with alpha tocopherol: (*a*) Appearance sixty-three days after caponizing. (*b*) Improvement one week after the first injection of testosterone propionate.

COMMENT

The results of this experiment appear to show that in male castrated birds the effectiveness of injected testosterone propionate is dependent on an adequate supply of vitamin E in the diet. Ordinarily in the normal bird there is a considerable excess of androgen over the amount necessary to maintain the secondary sex characters. This is evident from the fact that the secondary sex characters remain in a normal state for a considerable time after removal of the testes. Since this is so, any effect which vitamin E might have on the utilization of androgen would probably be effectively masked. By the removal of the testes and the establishment of a condition of severe hormone deficiency as well as of vitamin E deficiency it has been possible to study the relation of vitamin E to the effects of minimal amounts of androgen. The results indicate that a definite relation exists and also that a condition of severe vitamin E deficiency is necessary before the utilization of androgen becomes impaired. This inference is supported by the results obtained in group B in which the differences in response to injected testosterone propionate probably parallel differences in the degree of vitamin E deficiency.

It is well established [10] that a number of substances have a similar relation to the effectiveness of injected testosterone propionate. Thus, alcohols, acids and derivatives of certain fatty acids enhance the action of testosterone in the rat. Other substances, such as glycerol and paraffin, lower the action. On the basis of these observations the relation of vitamin E to the utilization of androgen is not a unique reaction but in the present instance it is highly significant.

SUMMARY

Caponized male fowl reared on a diet deficient in vitamin E were given injections of testosterone proprionate after a sufficient period had elapsed to allow regression of the male secondary sex characteristics. Four of the birds were given dietary supplements of alpha tocopherol. Four were maintained throughout the experiment on the original treated ration. Birds receiving both testosterone and alpha tocopherol responded to the androgen much more rapidly and effectively than those not receiving the vitamin E supplement. It is concluded, therefore, that in the fowl a certain level of vitamin E in the diet is necessary to insure the most effective utilization of androgen.

10. Allen, Danforth and Doisy,[8] p. 833.

CHOLESTEROL CONTENT OF BRAIN IN NUTRITIONAL ENCEPHALOMALACIA OF VITAMIN E-DEFICIENT CHICKS

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The brains of young chicks suffering from brain degeneration as a result of vitamin E deficiency exhibit occasionally an extraordinary edema. Since this condition is usually associated with some disturbance in cholesterol metabolism, and since brain tissue is a very rich source of cholesterol, it became desirable to determine whether or not any change in cholesterol content of the brain could be demonstrated in the diseased chicks. Accordingly, this aspect of the problem has been investigated, and the results are presented in this article.

METHOD OF ESTABLISHING VITAMIN E DEFICIENCY

The brain disease under investigation is apparently identical with the nutritional encephalomalacia described by Pappenheimer and Goettsch [1] in young chicks reared from the time of hatching on a synthetic vitamin E-free diet. In the present work, the disease appeared in groups of chicks on which studies of vitamin E deficiency were being conducted, but the diet used was a natural ration treated with ferric chloride in ether, after the procedure of Waddell and Steenbock,[2] to destroy any vitamin E it might contain. A slight modification in technical method was used, however, which proved to be quite significant. This involved the evaporation of ether *using heat*, a procedure contrasting with the original method of Waddell and Steenbock, in which the ether was allowed to evaporate spontaneously *without heat*. In the latter case brain degeneration does not occur—a result which has also been obtained by Pappenheimer and Goettsch. The food used in these experiments consisted of basal ration 152 composed of: ground yellow corn 46 parts, wheat bran 13, wheat middlings 13, alfalfa meal 5, meat scrap 10, dried skim milk 10, salt 1 part and cod liver oil 0.5 per cent. The food was soaked in an ether solution of ferric chloride (1 per cent by weight of the food) for a period of twelve hours or more, after which the ether was evaporated on a steam cone. The treated food was thoroughly mixed with a vitamin A-D supplement (Nopco [2a]) to replace

From the Department of Zoology, University of Illinois.

1. (*a*) Pappenheimer, A. M., and Goettsch, M.: J. Exper. Med. **53**:11, 1931. (*b*) Pappenheimer, A. M.; Goettsch, M., and Jungherr, E.: Nutritional Encephalomalacia in Chicks and Certain Related Disorders of Domestic Birds, Bulletin 229, Storrs, Conn., Agricultural Experiment Station, 1939.

2. Waddell, J., and Steenbock, H.: J. Biol. Chem. **80**:431, 1928.

2a. Nopco is the trade name of a blend of vitamins A and D with cod and other fish liver oils, sold by the National Oil Products Company, Harrison, N. J.

any A or D lost by the treating process. In various other experiments, cod, sardine and halibut liver oils have been used, and brain degeneration occurs with all of them. Curative and preventive experiments using good sources of vitamin E (concentrates made from wheat germ oil and, more recently, synthetic vitamin E, or alpha tocopherol) have given abundant evidence that vitamin E deficiency is involved in producing the condition.

EXPERIMENTAL PROCEDURE

Groups of newly hatched chicks, confined in wire-floored cages, were reared on the treated food usually for a period of six to eight weeks. During this time the condition of brain degeneration appeared somewhat sporadically, but with great suddenness, and was marked by imbalance, final prostration and death (Pappenheimer and Goettsch [1b]; Adamstone [3]). Autopsy revealed degenerate areas in the brain, particularly in the cerebellum, ranging from microscopic lesions to a condition of extensive disintegration. In the present study, the experimental chicks were observed carefully from day to day, and as soon as definite signs of illness appeared, were removed for investigation. In addition a group of normal controls reared on the untreated ration (basal ration 152) furnished material for the determination of the normal cholesterol content of the brain. In making such a determination the whole brain was used, although the cerebellum showed the most conspicuous lesions. This was done because the cerebrum is also visibly affected in some cases and probably contains microscopic lesions in many instances.

The sick and the normal chicks being studied were removed to the laboratory for convenience in handling. Each chick was killed by decapitation, and the head was carefully bled to remove as much free blood as possible. The roof of the skull was quickly removed, and the brain was excised by being cut free at the anterior end of the cerebral hemispheres, at the base of the spinal cord and at the base of the hypophysis. The wet brain was weighed and then carefully ground up with a known weight (7 Gm.) of plaster of paris in a clear glass mortar. A few drops of distilled water were added to insure solidification of the whole mass, after which it was evaporated to dryness in an oven at 42 C. The hard mass was then powdered again in the mortar and carefully scraped out on a piece of ether-extracted, weighed paper. A small piece of this paper held with forceps was used to wipe any remaining traces of material from the mortar and pestle. The weight of the powder along with the paper was again determined. Each sample was wrapped in the paper and bound with a piece of thin copper wire. Specimens were stored in a refrigerator until the cholesterol extractions could be made.

CHOLESTEROL DETERMINATIONS

In making the cholesterol determinations the powdered mixture was thoroughly extracted with chemically pure chloroform. Soxhlet extractors of 100 or 250 cc. capacity were used for this purpose. The extractors were set up the night before, so that the brain material was soaked in chloroform for about twelve hours before the actual extraction was carried out. Two hours' continuous extraction resulted in complete removal of cholesterol after the prolonged soaking. After extraction, the chloroform solution was cooled quickly, transferred to a volumetric flask and made up to 250 cc. volume by the addition of chemically pure chloroform. With 15 cc. of this solution, a Liebermann-Burchard test for cholesterol was carried out—

3. Adamstone, F. B.: Arch. Path. **31**:603, 1941.

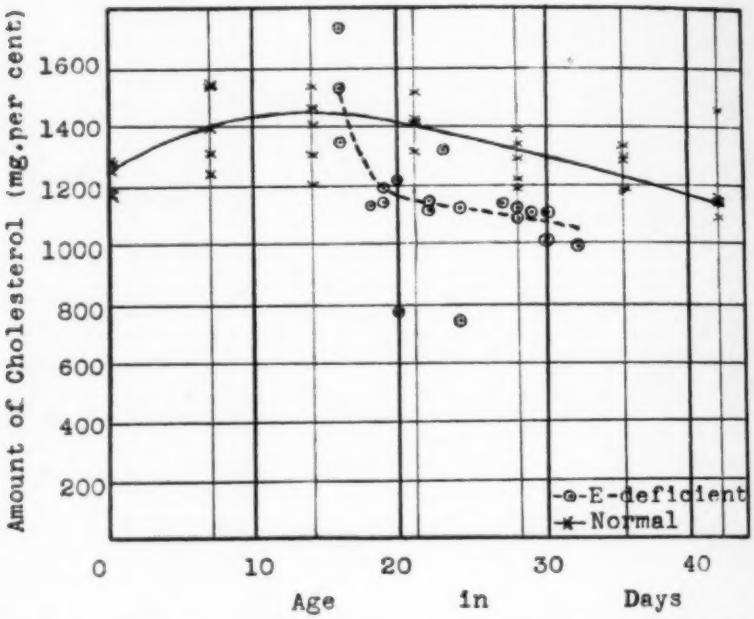
TABLE 1.—*Cholesterol Content of the Brains of Normal Chicks*

Chick	Age, Days	Weight, Gm.	Sex	Brain Weight		Cholesterol in Brain	
				Wet, Gm.	Dry, Gm.	Total, Mg.	Mg. per 100 Gm.
1	0	34	F	0.84	0.15	1.92	1,280
2	0	39	M	0.99	0.16	2.07	1,294
3	0	36	F	0.91	0.16	2.01	1,256
4	0	35	F	0.89	0.15	1.78	1,187
5	0	37	F	0.86	0.18	2.14	1,188
6	7	58	M	1.16	0.21	3.26	1,552
7	7	68	F	1.18	0.24	3.15	1,312
8	7	55	M	1.06	0.23	2.87	1,248
9	7	62	M	1.06	0.21	2.93	1,395
10	7	44	M	1.11	0.21	3.25	1,548
11	14	106	M	1.43	0.31	3.23	1,203
12	14	102	F	1.33	0.24	3.71	1,546
13	14	113	F	1.54	0.34	4.42	1,300
14	14	116	M	1.52	0.28	4.09	1,461
15	14	107	M	1.48	0.32	4.50	1,406
16	21	* 185	M	1.67	0.32	4.52	1,412
17	21	165	M	1.57	0.31	4.09	1,319
18	21	172	F	1.79	0.35	4.93	1,409
19	21	193	M	1.79	0.34	4.82	1,421
20	21	152	M	1.62	0.31	4.70	1,513
21	28	305	M	1.88	0.36	4.85	1,347
22	28	180	M	2.01	0.43	5.57	1,295
23	28	185	F	1.89	0.39	4.61	1,182
24	28	255	M	1.77	0.37	4.52	1,222
25	28	301	M	1.84	0.34	4.72	1,388
26	35	365	F	2.01	0.41	5.23	1,276
27	35	365	M	2.08	0.38	4.92	1,295
28	35	345	M	2.06	0.42	4.94	1,176
29	35	387	M	2.14	0.40	4.76	1,190
30	35	345	F	2.26	0.44	5.86	1,332
31	42	475	F	2.22	0.45	5.11	1,135
32	42	503	M	2.42	0.47	5.40	1,149
33	42	405	M	2.46	0.51	5.42	1,062
34	42	460	F	2.45	0.50	5.83	1,161
35	42	438	M	2.31	0.42	6.17	1,469

TABLE 2.—*Cholesterol Content of the Brains of Vitamin E-Deficient Chicks*

Chick	Age, Days	Weight, Gm.	Sex	Brain Weight		Cholesterol in Brain	
				Wet, Gm.	Dry, Gm.	Total, Mg.	Mg. per 100 Gm.
1	16	100	M	1.48	0.22	3.37	1,532
2	16	98	M	1.44	0.21	3.60	1,714
3	16	82	M	1.42	0.25	3.37	1,348
4	18	104	M	1.43	0.28	3.12	1,114
5	19	97	F	1.70	0.26	3.10	1,192
6	19	142	M	1.62	0.33	3.79	1,148
7	20	123	M	1.54	0.36	2.82	783
8	20	155	M	1.94	0.34	4.15	1,221
9	22	131	F	1.72	0.31	3.55	1,145
10	22	126	M	1.68	0.28	3.12	1,114
11	23	117	M	1.80	0.27	3.55	1,315
12	24	122	F	1.67	0.30	3.35	1,117
13	24	114	M	1.54	0.36	2.67	741
14	27	192	F	2.07	0.41	4.66	1,136
15	28	160	F	1.60	0.23	2.57	1,117
16	28	225	M	1.75	0.32	3.95	1,097
17	29	168	M	1.87	0.39	4.30	1,103
18	30	250	F	1.99	0.34	3.75	1,100
19	30	244	M	1.81	0.36	3.62	1,006
20	32	240	F	1.85	0.31	3.05	984

the color being developed in the dark for ten minutes. After this had been done, a photelometer reading was taken for the sample under consideration, using a standard Cenco Sheard photelometer. By interpolation on a calibration curve, previously determined with the photelometer for cholesterol solutions of known concentration, the cholesterol concentration of each brain extract was determined. The total cholesterol content, including cholesterol esters, could then be readily determined, and since dry weight of the brain was known, the cholesterol concentration in milligrams per hundred grams of dry brain could be calculated. This was done for chicks suffering from encephalomalacia as well as for the groups of normal chicks which had been removed, 5 weekly, for the purpose. The data



Graph illustrating the amount of cholesterol in the brains of normal and vitamin E–deficient chicks.

obtained from this experiment are tabulated in table 1 (normal brains) and table 2 (vitamin E–deficient brains) and illustrated by the chart.

Table 1 shows that there is a progressive increase in the total cholesterol content of the normal brain with age. In the E–deficient brains on the other hand (table 2) there is little if any increase in total cholesterol content with age. The chart shows that in the normal chick the amount of cholesterol in milligrams per hundred grams of dry brain substance increases slowly until the beginning of the third week and then diminishes slowly to the end of the sixth week, at which time the experi-

ment was discontinued. In the diseased chicks, by contrast, the cholesterol content in milligrams per hundred grams of dry brain at sixteen days is at the normal level. In succeeding days there is a rapid decrease, so that the amount of cholesterol in milligrams per hundred grams of dry brain substance falls considerably below that of the normal chick.

COMMENT

A consideration of the findings presented suggests that during the very early life of the newly hatched chick which is receiving a ration deficient in vitamin E the normal metabolic activity with respect to cholesterol is maintained for about two weeks. At this time, presumably, a sudden derangement occurs, after which the cholesterol content of the brain falls rapidly below the normal level. Beginning at this time, the condition of brain degeneration sets in, and some chicks show an immediate reaction, while others show no recognizable symptoms for some time or escape the disease altogether. This outcome is probably dependent on the region of the brain which is first affected and also on the amount of vitamin E available.

Similar studies of the cholesterol content of the blood and of various tissues of rabbits suffering from nutritional muscular dystrophy were carried out by Morgulis and Spencer.[4] They reported a very great increase in the cholesterol content of the skeletal muscles, slight increases in the visceral muscles, kidney and brain and a decrease in the lung, liver and spleen. Recovery was followed by a subsequent return to normal. This is just the opposite of the results obtained for the brains of chicks suffering from nutritional encephalomalacia, but the diseases are very different. In any event, it is evident that there is a pronounced derangement of cholesterol metabolism in both diseases associated with vitamin E deficiency. Vogt-Möller[5] reported hypocholesteremia in pregnant women subject to habitual abortion. The condition was improved following administration of vitamin E.

SUMMARY

Determinations of the total cholesterol content of the brain have been carried out for normal chicks and vitamin E–deficient chicks in which nutritional encephalomalacia had developed. The brains were extracted with chloroform, and cholesterol was determined colorimetrically with a photelometer, the Liebermann-Burchard reaction being used.

4. Morgulis, S., and Spencer, H. C.: J. Nutrition **12**:173, 1936.

5. Vogt-Möller, P.: Klin. Wchnschr. **15**:1883, 1936; abstracted in Alpha-Tocopherol (Vitamin E): Annotated Bibliography, Rahway, N. J., Merck & Company, Inc., 1939.

The results of this experiment show that the normal brain contains a higher proportion of cholesterol in milligrams per hundred grams of dry brain substance than the brains of chicks suffering from nutritional encephalomalacia. The decrease in cholesterol content of the brains of diseased chicks occurs during the third week, progressing rapidly thereafter. It is suggested, therefore, that this time marks a critical period for the maintenance of normal cholesterol metabolism in the brain of the chick. Furthermore, since a cholesterol disturbance also occurs in dystrophic rabbits and in women subject to habitual abortion, there appears to be a very definite relation between vitamin E and normal cholesterol metabolism.

It has also been demonstrated that in the brains of chicks deficient in vitamin E there is little or no increase in cholesterol content after the second week, whereas there is a marked increase in the normal brain. This fact gives further support to the possibility of a relationship between vitamin E and cholesterol metabolism.

RETICULUM CELL SARCOMA FOLLOWING ULCERATION OF THE INTESTINE IN VITAMIN E–DEFICIENT CHICKS

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In a previous article,[1] a lymphoblastoma which occurred in young chicks reared on an iron-treated, vitamin E–deficient ration was described. In a considerable number of these chicks as well as in specimens from other groups reared in the same manner, ulcers developed in the lower part of the intestine, followed by extensive tumor growths. These tumors were apparently very similar in some ways to the lymphoblastoma previously described, and since subsequent experiments involving changes in the vitamin A-D supplement appear to furnish a more definite clue to their cause, they are described in detail in this report.

EXPERIMENTAL METHODS

The chicks used in these experiments were white Leghorns of the same stock as those used throughout this work. They were reared from the time of hatching in wire-floored brooder pens and fed on ration 152.[2] This was composed of natural foodstuffs treated with a solution of ferric chloride in ether to destroy vitamin E. It is important, however, to point out that in the original experiments, in which these intestinal lesions were encountered, a vitamin A-D supplement in the form of cod liver oil or sardine oil was added to the treated food. In the latter experiments the vitamin A-D supplement was changed to halibut liver oil, but no intestinal lesions were found in these chicks, although 98 of them lived beyond three months. By this time, the lesions are usually well developed.

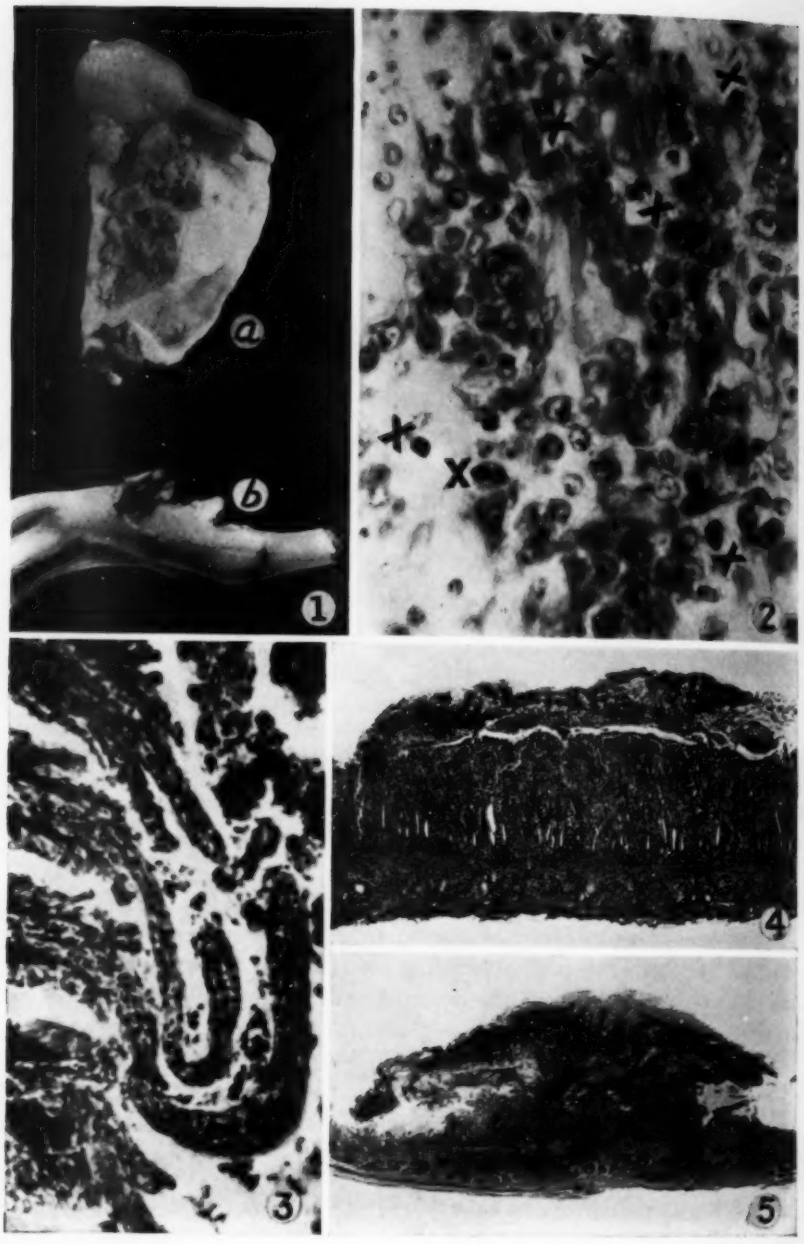
GROSS AND MICROSCOPIC APPEARANCE OF THE LESIONS

The lesions were always confined to the lower part of the intestine or to the rectum, sometimes extending into the ceca or for a short distance into the colon. They could be detected through the wall of the intestine because of the grayish discoloration and the presence of extensive blood clots. They consisted of irregularly flattened foliate expansions of tissue, projecting into the lumen of the tube and spreading over the inner surface (fig. *1 a*). In cross section the older and more advanced

From the Department of Zoology, University of Illinois.

1. Adamstone, F. B.: Am. J. Cancer **28**:540, 1936.

2. The content of this ration was ground yellow corn 46 parts, wheat bran 13, wheat middlings 13, alfalfa meal 5, meat scrap 10, dried skim milk 10, salt 1 and cod liver oil 2. In treating the food, a solution of ferric chloride (1 per cent by weight of the food) was used. The vitamin A-D supplement was added after treatment.



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growths were fungoid in shape, having a rounded surface with overhanging edges and a broad base or stalk connected to the wall of the intestine (fig. *5*). They varied in size from minute projections to masses more than 1 cm. in diameter. Ordinarily they were quite numerous and sometimes covered almost the entire inner surface of the intestine, apparently having fused as they increased in number and size. In the majority of cases the growths were confined to the lumen of the tube, but in 2 instances the wall of the intestine was completely perforated (fig. *1 b*).

The steps in the development of these growths were readily followed in gross material and in sections. For this study, 18 specimens have been used. Some of these show the beginnings of tumor masses and reveal a general ulcerating condition of the lower part of the intestine. Sections show that the epithelium of the exposed surface of the villi degenerates, followed by erosion of the epithelial cells. The free ends of the villi thus become converted into open tubes of epithelium with exposure of the inner core of reticular connective tissue. This reticular tissue begins to undergo rapid proliferation, and numerous mitotic figures may be found in it (fig. *2*). Ordinarily mitotic figures are fairly abundant in the epithelium, but they are not common in the deeper layers of the intestine. As a result of the cellular increase, the cores of the villi become much broader than usual, or they may become greatly elongated and protrude from the open ends of the villi as long finger-like projections (fig. *3*). As epithelial erosion becomes more extensive, these structures fuse to form the flattened tumor masses extending over neighboring villi (fig. *4* and *5*). In some cases the growth may be so extensive as to fill the lumen of the digestive tube, or it may grow deeper into the wall of the intestine, invading the underlying layers of tissue, including the submucosa, the muscularis and the serous layer.

EXPLANATION OF FIGURE

1, photograph showing (*a*) a portion of the rectum opened to disclose tumor masses and (*b*) a tumor mass protruding into the celom through the perforated wall of the rectum.

2, numerous mitotic figures in the reticular connective tissue core of a villus; × 495.

3, excessive growth of reticular connective tissue of villi, producing long finger-like projections through the eroded ends of the villi into the lumen of the intestine; × 113.

4, cross section of a young tumor; × 24.

5, cross section of an older, well developed tumor; × 6.4. Invasion of the lower layers of the intestinal wall is apparent, as well as myxomatous degeneration of superficial tissues.

The normal tissues are thus eroded and destroyed, and the tumor may eventually perforate the wall of the intestine and protrude into the celom (fig. 1 *b*). Some infiltration of lymphocytes usually occurs, but this is not as extensive as was found in the earlier studies of the lymphoblastoma affecting the visceral organs.

DIAGNOSIS

It is apparent from their method of formation that the growths are composed of typical reticular connective tissue. This can readily be seen near the base of the tumor mass; a gradual transition occurs toward the outer exposed surface, where extensive myxomatous degeneration takes place, accompanied by bacterial invasion. Hemorrhage is also common, due to the rupture of blood vessels. It is evident, therefore, that this growth has the invading and destructive effects of a true malignant tumor, and because of its location in the intestine, it must also produce very serious injury to the general health of the chick. Taking this into consideration, as well as the fact that it arises from reticular connective tissue, the growth may be appropriately designated as a reticular cell sarcoma.

COMMENT

The occurrence of tumor growths of this type is of considerable interest in view of the fact that they were not found in chicks which had been fed halibut liver oil as the vitamin A-D supplement in place of cod liver oil or sardine oil. Obviously, they are a direct result of the extensive development of ulcers in the intestine. Hence it appears that the use of cod liver oil or sardine oil in conjunction with the iron-treated, vitamin E–deficient diet is associated in some way with the production of both ulcers and tumors. The visceral organs of these chicks were also affected by extensive tumor growths,[1] but this fact was not at first associated with the use of cod liver or sardine oil. In later experiments, however, in which halibut liver oil was used, an entirely different reaction occurred.[3] This involved extensive erythrophagocytosis within the sinusoids of the liver and deposition of the hemosiderin in the hepatic cells with production of degenerative changes. The tumor growths in the visceral organs of chicks receiving cod liver oil developed mainly by proliferation of the reticular tissue which forms the endothelial linings of the blood vessels. This served as the stroma of the tumor, but the main mass of it was composed of heavy accumulations of rapidly multiplying lymphocytes. These growths were diagnosed as true neoplasms since they exhibited the characteristic properties of tissue destruction and infiltration. They were also readily transplantable, and when transplanted, produced similar reactions in the host.

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3. Adamstone, F. B.: Arch. Path. **31**:613, 1941.

SUMMARY

The tumors described in this report appear to be true malignant growths. They are developed from the reticular connective tissue which forms the cores of the intestinal villi and the tunica propria of the wall of the digestive tube. They arise as a direct result of the occurrence of intestinal ulcers. As to the cause of their formation, one point appears clear, namely, that they have been produced only in those groups of experimental chicks which had a vitamin A-D supplement in the form of cod liver oil or sardine oil in their diet. The suspicion seems well founded, therefore, that the ingestion of these oils in combination with the iron-treated, vitamin E-deficient diet is related in some way to the production of the tumor growths. It remains to be seen, therefore, whether this development of tumors is due to the lack of some necessary substance in these oils or whether there is a disturbance in the utilization of vitamin D supplied in the form of cod liver oil or sardine oil under conditions of vitamin E deficiency.

RELATION OF VITAMIN E TO SUBSTANCES OF THE ANTHRACENE GROUP

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The occurrence of vitamin E and its role in reproduction and in the maintenance of normal conditions in the reproductive system of the rat were established by Evans,[1] Sure [2] and the early workers in the field. Investigators then turned their attention to the results of vitamin E deficiency in other animals. It soon became apparent that the reproductive organs are not the only structures affected, since a whole series of reactions were encountered. Several excellent summaries of these are already available, notably: Evans [3] (1932), Mattill [4] (1938), Mason [5] (1939, reproductive system), Vogt-Möller [6] (1940), John [8] (1939) and the Vitamin E Symposium of the Food Group of the English Society of Chemical Industry [8] (1940). It is unnecessary, therefore, to enter on a lengthy discussion of these effects, but a summary is given in the accompanying table.

ROLE OF VITAMIN E

The occurrence of such a variety of effects soon led to speculation concerning the role of vitamin E in the metabolism of the organism. One of the earliest explanations suggested was that vitamin E might be concerned with the utilization of iron, but Mattill [9] rejected this suggestion after a careful review of the subject. Mason [10] was impressed

From the Department of Zoology, University of Illinois.

1. Evans, H. M.: Mem. Univ. California **8**:116, 1927.

2. Sure, B.: Dietary Requirements for Fertility and Lactation: I. The Rôle of Fat Soluble Vitamins in Fertility and Lactation, Bulletin 250, University of Arkansas, College of Agriculture, 1930.

3. Evans, H. M.: J. A. M. A. **99**:469, 1932.

4. Mattill, J. A.: J. A. M. A. **110**:22, 1938.

5. Mason, K. E., in Allen, E.; Danforth, C. H., and Doisy, E. A.: Sex and Internal Secretions, Baltimore, Williams & Wilkins Company, 1939.

6. Vogt-Möller, P., in Vitamin E: A Symposium, New York, The Chemical Publishing Company, 1940.

7. Drummond, J. C., in Vitamin E: A Symposium, New York, The Chemical Publishing Company, 1940.

8. John, W.: Ergebn. d. Physiol. **42**:1, 1939.

9. Mattill, J. A.: J. A. M. A. **89**:1505, 1927.

10. Mason, K. E.: J. Exper. Zool. **45**:159, 1926; footnote 5.

by the fact that the effects of vitamin E deficiency are often first apparent in structures characterized by rapid cell division, such as the developing embryo or the testis. He suggested, therefore, that this vitamin has some role in maintaining normal cell division. Vogt-Möller,[6] impressed by the same facts, stated that "there appears to be sufficient evidence for assuming that vitamin E acts directly or indirectly as a sort of morphogenic hormone." Drummond [7] and Underhill [11] emphasized the possibility of hormonal imbalance, particularly as it affects the pituitary body. While such an explanation may be satisfactory enough in relation to certain reactions, it is of little value in reference to muscular dystrophy or to nutritional encephalomalacia in the chick, for example. John,[8] reviewing the subject largely from the chemical aspect, suggested that the vitamin has a role in the oxidation mechanism of the organism. As yet, however, there is no evidence concerning this possibility. It appears, in view of the great variety of reactions encountered, that most of the explanations so far put forward are too narrow, since in general they can explain only one or two effects of the deficiency. The possibility of still another but considerably broader interpretation is presented in this paper as an outcome of experimental work carried out with the chick.

The program of research on which this interpretation rests was begun some ten years ago with the object of finding out how the chick would respond to vitamin E deficiency. From the table it is apparent that a considerable variety of reactions have occurred in this animal, just as in the rat. These have been studied as thoroughly as facilities would permit, and a tentative analysis of the results may therefore be permissible.

In setting up the condition of vitamin E deficiency in these experiments, use was made of the discovery by Waddell and Steenbock [12] (1928) that treating a natural ration with ferric chloride in ether would effectively inactivate or destroy the vitamin E in the food. This made it possible to employ a ration composed of natural foodstuffs rather than a synthetic diet, which is difficult to use with the chick. Since Waddell's original method involved evaporation of the ether in the open air, there was entailed the loss of considerable quantities of ether and the exposure of the food to rapid oxidation. In order to overcome these difficulties, the procedure was slightly modified so that the food was treated in a series of closed glass containers and the ether evaporated off with heat and condensed. The use of this method has given a number of results which could not have been obtained otherwise and which apparently throw considerable light on the role of vitamin E.

11. Underhill, S. W. F., in Vitamin E: A Symposium, New York, The Chemical Publishing Company, 1940.

12. Waddell, J., and Steenbock, H.: J. Biol. Chem. **80**:431, 1928.

Reported Effects of Vitamin E Deficiency in Experimental Animals

Animal	Death of Embryo	Injury to Female Reproductive System	Deciduomas	Testicular Degeneration	Paralysis of Young	Nervous Disorders of Adults	Muscular Dystrophy	Myopathy of Gizzard	Thyroid Dysfunction	Hypophysis Dysfunction	Brain Degeneration	Lymphoblastoma	Erythrophagocytosis	Retardation of Growth Rate	Author *
Rat....	+	..	+	+	+	..	+	..	..	..	..	..	..	+	Evans, H. M.: Proc. Nat. Acad. Sc. **11**: 373, 1925; Am. J. Physiol. **85**: 149, 1928; J. Nutrition **1**: 23, 1928. Evans, H. M., and Burr, G. O.: J. Biol. Chem. **76**: 273, 1928. Evans, H. M.; Emerson, G. A., and Telford, I. A.: Proc. Soc. Exper. Biol. & Med. **38**: 625, 1938. Evans.[1]
Rat....	+	..	..	..	.	..	..	..	..	..	..	..	..	+	Sure, B.: J. Biol. Chem. **58**: 693, 1924.
Rat....	+	..	..	+	+	..	..	..	..	..	..	..	..	+	Mason, K. E.: Biochem. J. **32**: 1785, 1938. Mason.[10]
Rat....	+	..	..	+	..	..	..	..	..	..	..	..	..	..	Waddell and Steenbock.[12]
Rat....	+	..	..	+	..	..	..	..	..	..	..	..	..	..	Kudrjaschov, B. A.: Endokrinologie **7**: 91, 1930.
Rat....	+	.	..	..	..	..	..	..	..	..	..	..	..	..	Vogt-Möller.[6]
Rat....	..	..	..	..	..	..	..	..	..	+	..	..	..	..	Van Wagenen, G.: Anat. Rec. **29**: 398, 1925.
Rat....	..	..	..	..	..	..	..	..	..	+	..	..	..	..	Nelson, W. O.: Anat. Rec. **56**: 241, 1933.
Rat....	+	..	..	..	+	..	..	..	+	+	..	..	..	..	Barrie, M. M. O.: Lancet **2**: 251, 1937; Nature, London **139**: 286, 1937; Biochem. J. **32**: 1474, 1938. Singer, E.: J. Physiol. **87**: 287, 1936.
Rat....	+	+	..	..	..	..	..	..	..	+	..	..	..	..	Bacharach, A. L.; Allcome, E., and Glynn, H. E.: Biochem. J. **31**: 2287, 1937. Weisner, B. P., and Bacharach, A. L.: Nature, London **140**: 972, 1937.
Rat....	+	+	..	..	..	..	+	..	..	..	..	..	..	..	Martin, A. J. P., and Moore, T.: J. Hyg. **39**: 643, 1939.
Rat....	..	..	..	..	..	+	+	..	..	..	..	..	..	..	Einarson, L., and Ringsted, A.: Effect of Chronic Vitamin E deficiency on the Nervous System and the Skeletal Musculature in Adult Rats, Copenhagen, Levin & Munksgaard, 1938.
Rabbit	..	..	..	..	..	..	+	..	..	..	..	..	..	..	Goettsch, M., and Brown, E. F.: J. Biol. Chem. **97**: 549, 1932. Madsen, L. L.: J. Nutrition **11**: 471, 1936. Morgulis, S., and Spencer, H. C.: ibid. **11**: 573, 1936. Knowlton, G. C., and Hines, H. M.: Proc. Soc. Exper. Biol. & Med. **38**: 665, 1938. Mackenzie, C. G., and McCollum, E. V.: Science **89**: 370, 1939.
Guinea pig	..	..	..	..	..	..	+	..	..	..	..	..	..	..	Goettsch, M., and Pappenheimer, A. M.: J. Exper. Med. **54**: 145, 1931.

* Merck's Annotated Bibliography,[18] although available only in mimeographed form, contains all the references given here (and many more), with the exception of Martin and Moore.

Reported Effects of Vitamin E Deficiency in Experimental Animals—Continued

Animal	Death of Embryo	Injury to Female Reproductive System	Deciduomas	Testicular Degeneration	Paralysis of Young	Nervous Disorders of Adults	Muscular Dystrophy	Myopathy of Gizzard	Thyroid Dysfunction	Hypophysis Dysfunction	Brain Degeneration	Lymphoblastoma	Erythrophagocytosis	Retardation of Growth Rate	Author *
Duck...	..	..	..	..	..	..	+	..	..	..	..	..	..	..	Pappenheimer, A. M., and Goettsch, M.: J. Exper. Med. **59**: 35, 1934.
Turkey	..	..	..	..	..	..	..	+	..	..	..	..	..	..	Jungherr, E., and Pappenheimer, A. M.: Proc. Soc. Exper. Biol. & Med. **37**: 520, 1937.
Chick..	..	..	..	..	..	..	..	..	..	..	+	..	..	..	Pappenheimer, A. M., and Goettsch, M.: J. Exper. Med. **53**: 11, 1931. Pappenheimer, A. M.; Goettsch, M., and Jungherr, E.: Nutritional Encephalomalacia in Chicks and Certain Related Disorders of Domestic Birds, Bulletin 229, Storrs, Conn., Agriculture Experiment Station, 1939.
Chick..	+	..	..	..	..	..	..	..	..	..	..	..	..	..	Barnum, G. L.: J. Nutrition **9**: 621, 1935.
Chick..	+	..	..	+	..	..	..	..	..	..	+	+	+	+	Adamstone, F. B.: J. Morphol. & Physiol. **52**: 47, 1931. Adamstone, F. B., and Card, L. E.: ibid. **56**: 339, 1934. Adamstone, F. B.: Am. J. Cancer **28**: 540, 1936.

RELATION OF ANTHRACENE COMPOUNDS TO STRUCTURES AFFECTED BY VITAMIN E DEFICIENCY

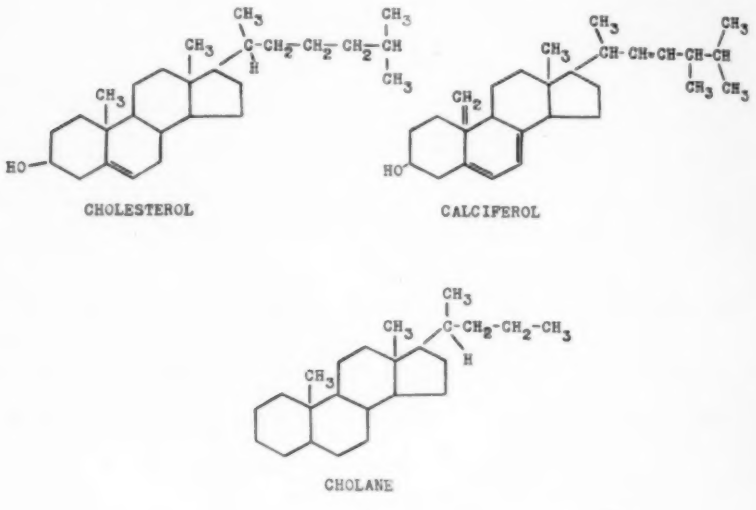
In the presentation of this interpretation it seems best to develop it in the order in which it originally took shape. If reference is made to the chart of anthracene compounds (chart), an interesting parallel becomes evident between the substances listed, or the structures known to be good sources of them, and the reactions which are produced by vitamin E deficiency, as follows:

Cholesterol.—This substance is abundant in the brain, and brain degeneration, or nutritional encephalomalacia, occurs regularly in young chicks reared on a ration deficient in vitamin E. This suggests a possible derangement of cholesterol metabolism.

Calciferol (vitamin D).—The use of vitamin D in the form of cod liver oil or sardine liver oil as a supplement to the treated diet is accompanied by a very different reaction in the liver of the chick as compared with that which occurs when halibut liver oil is used. Thus the use of cod liver oil was accompanied by the development of lympho-

blastoma in young chicks (Adamstone [13]), whereas the use of halibut liver oil was followed by erythrophagocytosis and deposition of the hemosiderin in the hepatic cells (Adamstone [14a]). This suggests that vitamin D in different forms is utilized differently under conditions of vitamin E deficiency.

Androgen.—Androgen is associated with the testis, and this structure undergoes degeneration in vitamin E deficiency. This suggests that disturbances in proper utilization of androgen might be involved.



Cholane and related substances of the anthracene group which are intimately associated with structures affected by vitamin E deficiency.

Estrogens Progesterone.—These substances are associated with the female reproductive system, which is more or less seriously affected. This is more apparent in the rat than in the chick. In this case also some derangement in the action of these substances might be implied.

13. Adamstone, F. B.: Am. J. Cancer **28**:540, 1936.

14. Adamstone, F. B.: (*a*) Arch. Path. **31**:613, 1941; (*b*) this issue, p. 711; (*c*) this issue, p. 706.

The question may be raised, therefore: Does any relation exist between the effective normal utilization of the substances of the anthracene group and vitamin E?

A certain amount of evidence was already available concerning this point, namely, that the reaction of the liver in the chick to vitamin D in different forms varied according to the type of oil supplement used. It became necessary, therefore, to obtain more evidence bearing on this question. Accordingly two experiments were carried out, as follows:

EXPERIMENT 1.—The object of this experiment was to determine the amount of cholesterol in the brains of vitamin E–deficient chicks suffering from brain degeneration and to compare this amount with the similarly determined amount in the brains of normal chicks. The results of this experiment, given elsewhere (Adamstone [14b]), showed clearly that in normal chicks the amount of cholesterol in milligrams per hundred grams of dry brain substance showed a slight increase during the first three weeks followed by a slow decrease thereafter to the end of the sixth week, when the experiment was terminated. In the diseased chicks, by contrast, there was a normal cholesterol content at the time the disease first appeared in the third week followed by a sudden and continuing decrease below the normal. This was interpreted as indicating that cholesterol metabolism remained normal until the early part of the third week, when a sudden derangement occurred, accompanied by the onset of brain degeneration. Hence normal cholesterol metabolism appears to depend on an adequate supply of vitamin E.

EXPERIMENT 2.—The object of this experiment (Adamstone [14c]) was to determine the relation of vitamin E to the ability of male fowl to utilize androgen effectively. Injections of testosterone propionate were made in three groups of caponized males reared as follows: group *A*, on a normal diet; group *B*, on a diet deficient in vitamin E; group C, on a vitamin E–deficient diet which was supplemented with synthetic vitamin E, or alpha tocopherol, at the time the injections of the androgen were made. In birds of groups A and C there was an immediate and rapid response to the injections, the head furnishings not only increasing in size but becoming firm, erect and bright red. In the other group lacking vitamin E, the reaction to testosterone was much slower, and 2 birds gave little response. Hence by removing the testes and setting up a condition of severe hormone deficiency as well as vitamin E deficiency it has been possible to study the relation of vitamin E to the utilization of minimal amounts of an androgen. This experiment is interpreted as indicating that utilization of androgen is impaired when a condition of vitamin E deficiency is present.

COMMENT

The two experiments described have given results which along with those mentioned in connection with the use of various fish liver oils appear to justify a different interpretation of the role of vitamin E, namely: that this vitamin is necessary for normal and efficient utilization of chemical substances of the anthracene group, just as vitamin D is necessary for proper utilization of calcium and phosphorus. Moreover, substances of the anthracene group, including cholesterol, ergosterol, calciferol (vitamin D), androgen, estrogen and progesterone, all

have a basic chemical structure in that they are derivatives of the cholane base. This fact would appear to be highly significant.

It appears desirable now to consider as briefly as possible how the experimental work which has been done with other animals gives support to the explanation which has been given.

1. The occurrence of paralysis and nervous disorders in rats recalls the effects of brain degeneration in chicks. Paralysis is now known to be definitely associated with muscular dystrophy (see next paragraph) and some disturbance in the cholesterol content of both muscular and nervous tissues is probably involved. Some suggestion of this might be inferred from the work of Watson (quoted by Sugita [15]), which shows that myelination of the cerebral cortex of the rat proceeds most actively between 10 and 35 days of age. This includes the age at which paralysis of suckling young occurs.

2. Muscular dystrophy in the rat, rabbit and guinea pig appears to be definitely related to vitamin E deficiency, although some other factor is thought to be involved. It is noteworthy that Madsen [16] suggested a possible relation between vitamin E deficiency, the use of a cod liver oil supplement and muscular dystrophy.

Other studies on muscular dystrophy in the rabbit carried out by Morgulis and Spencer [17] have demonstrated a severe disturbance in cholesterol metabolism, which is indicated by a very great increase in the cholesterol content of the voluntary muscles and certain other structures. These results appear to be extremely significant.

3. Vogt-Möller [18] noted the occurrence of hypocholesteremia in pregnant women who were subject to habitual abortion. He found that the hypocholesteremia was favorably influenced by administration of vitamin E.

4. Testicular degeneration in the rat is accompanied by the appearance of castration cells in the hypophysis, although the interstitial cells apparently remain normal. This suggests ineffectual use of androgen or some derangement in the hormone. Hence, in view of the reciprocal relationship which exists between the gonad and the anterior lobe of the hypophysis (Smith [19]), it is conceivable that a cycle of reactions is set up whereby ineffectual use of androgen has a harmful effect on

15. Sugita, N.: J. Comp. Neurol. **28**:511, 1917.

16. Madsen, L. L.: J. Nutrition **11**:471, 1936.

17. Morgulis, S., and Spencer, H. C.: J. Nutrition **12**:173, 1936.

18. Vogt-Möller, P.: Klin. Wchnschr. **15**:1883, 1936; abstracted in Alpha-Tocopherol (Vitamin E), Annotated Bibliography, Rahway, N. J., Merck & Company, Inc., 1939.

19. Smith, P. E., in Allen, E.; Danforth, C. H., and Doisy, E. A.: Sex and Internal Secretions, Baltimore, Williams & Wilkins Company, 1939.

the pituitary. This is reflected back on the testis in the form of decreased stimulation for the secretion of the hormone. Continued operation of this cycle becomes progressively more severe and eventually causes an effect similar to ablation of the anterior lobe of the hypophysis, namely, degeneration of the testis. This means, in other words, that maintenance of the testis is dependent on adequate secretion and proper ultilization of the secreted product and that failure to utilize the product effectively must eventually result in damage to the testis.

5. Attempts to explain the maternal share in failure to maintain pregnancy in the rat on the basis of estrogen and particularly progesterone have been unsuccessful (Vogt-Möller [6]). The present interpretation admits of such an explanation in terms of inability to utilize this hormone successfully.

Shute [20] suggested that vitamin E may be concerned in holding estrogen in equilibrium during pregnancy and that interruption of pregnancy in vitamin E deficiency may be due to an excess of estrogen in the blood. The same effect, namely, release of estrogen and subsequent abortion, would follow failure to utilize progesterone, since progesterone is an estrogen-inhibiting substance.

6. A number of investigators have reported experiments with tumor growths in relation to vitamin E. The results in general are quite contradictory. However, in view of the relation of the carcinogenic compounds to the anthracene group, it is not unreasonable to suppose that a definite relationship exists between vitamin E and tumor growth. For example, the development of a lymphoblastoma in vitamin E–deficient chicks receiving a cod liver oil supplement is suggestive of perverted metabolism of some chemical substances (in this case the vitamin D of cod liver oil), which under normal conditions may be very necessary to the well-being of the animal.

SUMMARY

A general survey of the extensive array of reactions to vitamin E deficiency which have been described by various investigators in different animals discloses that the structures affected by vitamin E deficiency are all more or less intimately associated with substances of the anthracene group. This suggests a broader interpretation of the role of vitamin E, particularly in regard to what it does in the organism: It is concerned with normal metabolism of some of the anthracene compounds. This conclusion is based on evidence from various studies relating to the utilization of cholesterol, vitamin D and androgen. These substances, as well as estrogen in various forms, progesterone and the

20. Shute, E.: J. Obst. & Gynaec. Brit. Emp. **44**:121, 1937.

carcinogenic compounds, are all derivatives of the cholane molecule—a fact which would appear to be highly significant.

If the correctness of this interpretation should be established, vitamin E would be elevated from the somewhat anomalous position it has held as the sterility vitamin to a place of considerable importance in the vitamin family. It must be remembered, however, that there is still no knowledge as to how vitamin E could carry out the role assigned to it. A stimulus to further investigation appears in the fact that the tocopherols are closely related to the antioxidants and that these substances, in turn, are intimately related to oxidative changes in fats and lipoidal substances. As yet, however, there is no evidence as to whether or not these substances retain their antioxidant properties when ingested into the animal organism.

PIGMENTED VILLONODULAR SYNOVITIS, BURSITIS AND TENOSYNOVITIS

A DISCUSSION OF THE SYNOVIAL AND BURSAL EQUIVALENTS OF THE TENOSYNOVIAL LESION COMMONLY DENOTED AS XANTHOMA, XANTHOGRANULOMA, GIANT CELL TUMOR OR MYELOPLAXOMA OF THE TENDON SHEATH, WITH SOME CONSIDERATION OF THIS TENDON SHEATH LESION ITSELF

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The yellow-brown tumor-like tenosynovial lesion which usually goes under the name of xanthoma, xanthogranuloma, giant cell tumor, xanthomatous giant cell tumor or myeloplaxoma of the tendon sheath is of fairly common occurrence and has received considerable attention in the medical literature. Even within the same tendon sheath lesion, the cytologic pattern usually presented is one in which very cellular areas, composed of closely compacted polyhedral cells and variable numbers of multinuclear giant cells and lipoid and hemosideric cells, alternate with areas in which intercellular collagen and hyalin largely crowd out the cells and dominate the picture. This pattern, which is familiar to all pathologists, represents a rather advanced stage in the evolution of the lesion. The earlier stages are not generally familiar and will be stressed here because of their importance for the understanding of the evolution not only of the tendon sheath lesion but of its anatomic equivalents in synovial membranes and bursae.

That the anatomic equivalent of this tendon sheath lesion does occasionally occur in the synovial membrane of a joint is fairly well recognized. Here the lesion may implicate only a small area (as it usually does in the tendon sheath) or the entire synovial membrane; i. e., the involvement may be circumscribed or diffuse. In the circumscribed form, the affected membrane shows one or more yellow-brown sessile or stalked tumor-like nodular outgrowths. In the diffuse form, the membrane appears brownishly pigmented and covered by villous and coarse nodular outgrowths (pigmented villonodular synovitis), though

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either villi or nodules may predominate in a given case. It is particularly the nodular outgrowths or projections that maintain the cytologic correspondence of these synovial lesions to the tendon sheath lesions. On this account, the circumscribed synovial lesions and those diffuse ones in which nodules are prominent or dominate are also often classified under the heading of xanthoma, giant cell tumor, and so on.

When a diffusely affected synovial membrane presents essentially a pigmented villous appearance, the condition is sometimes referred to as chronic hemorrhagic villous synovitis or arthritis. However, the cellular character of the sparse nodules which are nevertheless present in such cases relates these more villous lesions to the more nodular ones. On the other hand, should the nodular and villous projections have become fused and intergrown, as they sometimes have, a lesion of almost baffling cytologic complexity may evolve. To such a lesion, other names than those which have already been mentioned have sometimes been applied—for instance, "giant cell fibrohemangioma," "fibrohemosideric sarcoma," "sarcoma fusogigantocellulare" or "benign (or malignant) polymorphocellular tumor of the synovium." These varied and elaborate names alone would hardly lead one to suspect that basically the complex lesion was still related to the simpler villous or villonodular lesions.

As to analogous lesions in bursae, all that we wish to point out here is that a bursa may be the site of a pigmented villonodular lesion like that to be seen in the articular synovial membrane. This is not a very familiar fact, possibly on account of the special rarity of this type of lesion in bursae.

The total number of instances (counting both the circumscribed and the diffuse lesions) which have been reported to date of the condition we are calling pigmented villonodular synovitis is only about 57. Nevertheless, it is probably not as rare as this low figure suggests, for undoubtedly many cases remain unreported. Indeed, our own material comprises 20 cases, only 1 of which had been previously reported (Kling and Sashin[1]) and is included in the aforementioned total. Of these 20 cases, 11 showed the lesion in the diffuse and 9 showed it in the circumscribed form. However, the cases already published seem to indicate that the lesion is seen much more often (at least in a ratio of 2 to 1) in the circumscribed than in the diffuse form. It is also of interest that the lesion seems always to be monarticular. Furthermore, it seems to have been noted only in joints of the lower limbs, and, in particular, in the overwhelming majority of cases, in the knee joint.

We shall give, in this paper, an exposition of the synovial lesion in question, basing it mainly on our own 20 cases. In addition, the

1. Kling, D. H., and Sashin, D.: Arch. Surg. **30**:52, 1935.

analogous lesion in bursae mucosae will be briefly described on the basis of our study of 4 diffusely pigmented villonodular bursae. We shall give some consideration also to the so-called xanthoma, giant cell tumor, myeloplaxoma, etc., of tendon sheaths, on the foundation of the 55 instances recorded in our files. Among these instances, we were fortunate enough to find several which could still shed light on the manner of evolution of these tendon sheath lesions and which turned out consequently to be of importance in the integration of the entire subject.

In these pertinent tendon sheath specimens, some areas revealed that where the lesion was still actively evolving the tenosynovial lining was pigmented and viliously transformed and the sublining tissue showed focal collections of large roundish or polyhedral cells, which had apparently arisen through hyperplasia of undifferentiated (especially perivascular) connective tissue cells. In areas representing more advanced stages, the lesion could be seen to have grown by enlargement and fusion of individual nodules, between which synovium-lined clefts produced by invagination of the sheath lining could sometimes be seen. Furthermore, it could be demonstrated that it was the large round or polyhedral hyperplastic stromal cells that were becoming converted into the hemosideric or lipoid-bearing phagocytes or the multinuclear giant cells present in varying numbers in the larger nodules. In other words, these histologic features—precisely the ones which in the past have led to designation of this tendon sheath lesion as a xanthoma or a giant cell tumor, etc.—are created through modifications produced in the hyperplastic stromal cells by their acquisition of phagocytic qualities. Finally, it became clear that in areas where the active evolutionary phase had subsided there were collagenization and hyalinization of the stroma, crowding out the stromal cells.

Thus the tendon sheath specimens in which the evolution of the lesion could be traced did not only add to our understanding of the tenosynovial lesion by itself. They also served to link that lesion, in respect to evolution, with villonodular synovitis and bursitis by showing that the tenosynovial nodules develop in the same way, on the whole, as the synovial and bursal nodules. A point of difference worth noting, however, is that the tenosynovial nodules develop mainly outward (i. e., away from the tendon channel) while the synovial and bursal lesions develop mainly inward (i. e., toward the cavity).

We do not know what initiates the proliferative and hyperplastic processes creating such tenosynovial, synovial and bursal lesions. However, the acquisition of pronouncedly phagocytic qualities by the strategic hyperplastic stromal cells and the ultimate tendency toward their being

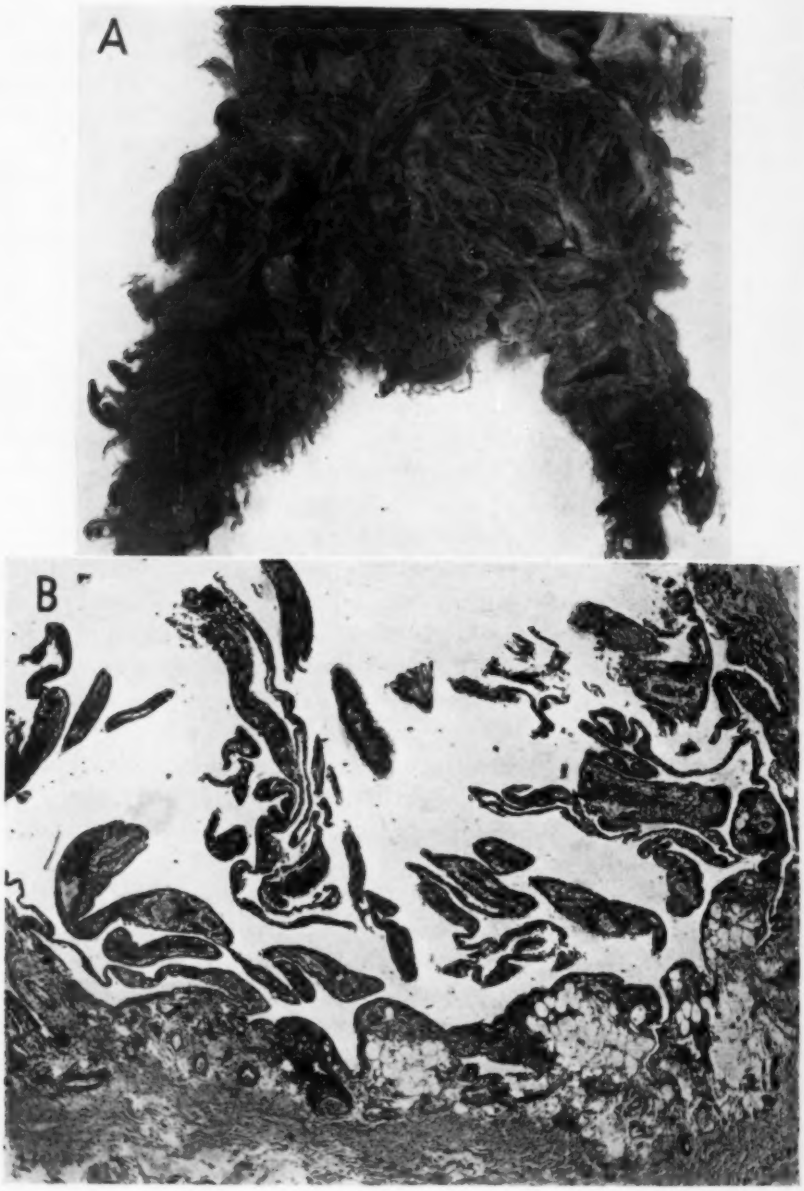

Figure 1

(See legend on opposite page)

crowded out by collagenization and hyalinization of the stroma suggest that the lesion in any of the three sites is based on an inflammatory rather than a neoplastic reaction. We have expressed this idea in using the ending "itis" in our designation of the lesion. It must be conceded, however, that we can offer no cue as to what the agent inciting this inflammatory reaction might be. It seems clear to us that it is not a bacterial one. Nor do we hold that the reaction represents simply a response to local lipoid imbalance or local repeated hemorrhage. At any rate, we feel confidence in maintaining that no more in tendon sheaths than in synovial membranes and bursal linings should the lesion be interpreted as a true tumor, holding, indeed, that it is only in a superficial clinical sense that the use of the term "tumor" in connection with these lesions seems justifiable at all.

PATHOLOGY OF PIGMENTED VILLONODULAR SYNOVITIS

Simon [2] described a clear example of the circumscribed type of the disorder as early as 1865, and Moser,[3] one of the diffuse type in 1909. Cases reported in the literature have been reviewed by Hartman,[4] Hunter,[5] De Santo and Wilson [6] and Galloway, Broders and Ghormley,[7] among others, and these various authors have also added cases of their own.

The already mentioned predilection of the lesion for the knee joint is apparent for instance from the report of De Santo and Wilson, who

2. Simon, G.: Arch. f. klin. Chir. **6**:573, 1865.
3. Moser, E.: Deutsche Ztschr. f. Chir. **98**:306, 1909.
4. Hartman, F. W.: Surg., Gynec. & Obst. **34**:161, 1922.
5. Hunter, W. C.: Am. J. Path. **7**:13, 1931.
6. De Santo, D. A., and Wilson, P. D.: J. Bone & Joint Surg. **21**:531, 1939.
7. Galloway, J. D. B.; Broders, A. C., and Ghormley, R.: Arch. Surg. **40**: 485, 1940.

EXPLANATION OF FIGURE 1

A, photograph of a knee joint synovial membrane which is thickly covered by long, tangled villi giving it, over much of its surface, the appearance of a straggly beard. Near the lower right hand corner of the picture (where the cross incision can be seen) the surface presents a smoother appearance, due to matting down of the villi, and in this area the synovial membrane has the texture of spongy tissue. The specimen comes from a 22 year old man in whom over a two year period the knee joint in question showed persistent effusion, recurring promptly after each of many tappings. In the history given by the patient, it was alleged that the swelling of this knee began just after an injury to it. The injury, however, seems to have been at most a mild and indirect one.

B, photomicrograph (× 30) of a section through a villous area of the synovial membrane pictured in *A*. Though cytologic details cannot be seen with this magnification, one can note the general character and conformation of the pigmented villi and some villous connecting strands between them.

collected 32 acceptable cases and added 9 of their own. Among these 41 cases, a knee joint was involved in 36, an ankle joint in 4 and the joint between the navicular and second cuneiform bones in 1. Similarly, from the report of Galloway, Broders and Ghormley, who accepted 43 cases and added 5 of their own, it appears that, of the total 48 cases, the lesion was in a knee joint in 42, in an ankle joint in 3 and in some tarsal joint in 3. Thus, if one adds to their total the 9 cases studied by De Santo and Wilson, it appears that the lesion was in a knee joint in 51 of the 57 cases (including circumscribed and diffuse forms of involvement) covered by these two reports. As to the localization in our own 20 cases, a knee joint was affected in 18, a tibiotarsal joint in 1 and a calcaneocuboid joint in 1. The lesion in the tibiotarsal joint was circumscribed, while that in the calcaneocuboid joint was diffuse.

Furthermore, as already indicated, though our own cases were about equally divided between the circumscribed and the diffuse form of involvement (9 circumscribed, 11 diffuse), one gathers from the literature that the circumscribed form is encountered much more commonly than the diffuse.

Diffuse Form.—There is considerable variety in the appearance of the diffusely affected synovial membranes. A synovial membrane showing the lesion in a diffuse predominantly villous form (fig. 1 *A*) is of a rather uniform reddish brown color, usually shading down in some places to yellowish brown. For the most part, the villi are delicate, long, tangled and more or less matted together into a straggly beard, but an occasional stubby villus, rather resembling a nodule, can also be noted. The straggly villous appearance may dominate the impression created by such a synovial membrane as a whole. However, in some places, closer inspection may reveal folds or even pads of tissue, resulting apparently from closer matting together and flattening down of the villi. The padlike areas, when incised at right angles to the surface, may be found to be a centimeter or more in thickness and to present the appearance of a coarse-meshed sponge.

Microscopic examination of areas covered by a beard of matted thin villi shows (fig. 1 *B*) that the latter have a coating of synovial lining cells and that their supporting stroma contains, among other elements, a considerable number of blood vessels and much blood pigment. Indeed, the pigmentation is to be noted not only in the stroma of the villi but also in most of the lining cells, which contain granules of hemosiderin in their cytoplasm. In some places the lining is one cell thick and in others two or three cells thick. The vascular channels are thin walled. An occasional one is encircled by a cuff of small lymphocyte-like cells, though at this stage, if the channel is cuffed at all, it is more likely to be by pigment-bearing cells. The supporting connective tissue stroma of

the villi is loose in texture and contains, dispersed through it, a variable number of either polyhedral or more spindle-shaped cells, crowded with granules of brownish pigment. A study of numerous fields reveals that many of these pigment-bearing stromal cells are actually hemosiderin-bearing lining cells which have migrated inward. Others apparently represent hemosiderin-bearing phagocytes formed through hyperplasia of the perithelial and stromal connective tissue.

Illuminating in relation to the gross structure of the villous matting in the bearded areas is the fact that microscopically the cross strands

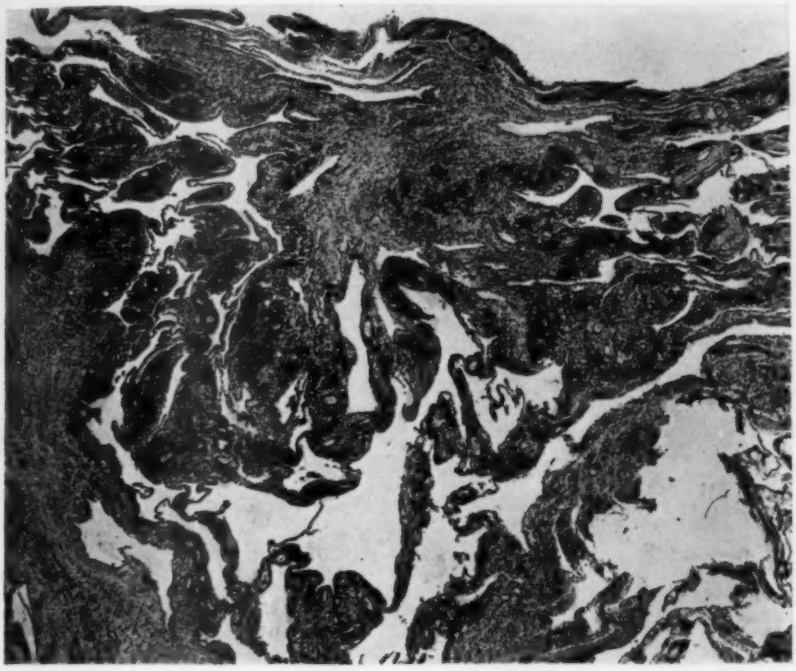

Fig. 2.—Photomicrograph (× 20) of a section through a spongy area of the synovial membrane pictured in figure 1 *A*. In some places the matted pigmented villi are separated only by narrow clefts, while in other places the spaces between them are much larger.

of tissue connecting the villi are, on the whole, so thin that in many places they consist merely of two apposed layers of synovial lining cells. An area which presents grossly a more spongy appearance (fig. 2) need not be essentially different microscopically from one of the more bearded areas, though showing richer and usually thicker webbing. Furthermore, the sparse stubby or nodular villi which one finds here and there in such cases approximate in their cytologic appearance the larger nodules which one sees in the more emphatically nodular synovial membranes.

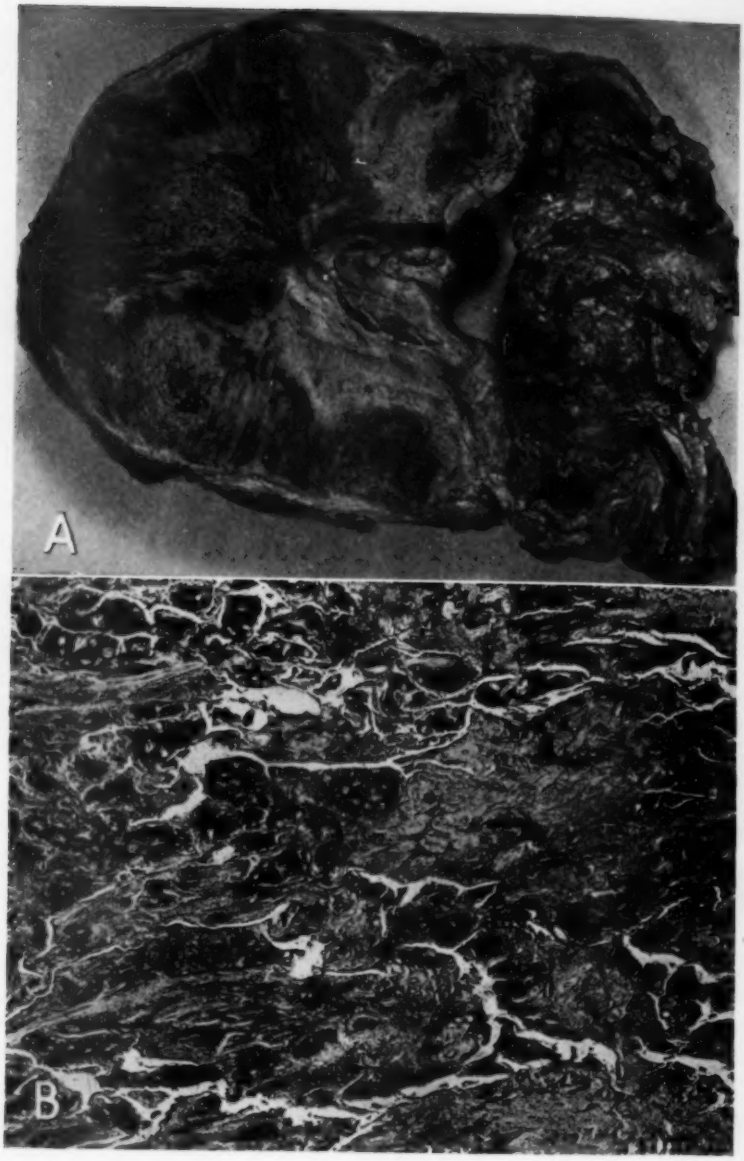

Figure 3

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Specifically, in addition to containing compact foci of polyhedral cells, these nodular villi may already present hemosiderin-bearing and lipoid-bearing foam cells and multinuclear giant cells and show some hyalinization of the stroma; i. e., they may present the same microscopic appearance as the nodules of the predominantly nodular synovial membranes and thus show their genetic relationship to the latter.

More complex gross and microscopic appearances are presented by the closely matted villous synovial membranes, in which fusion, intergrowth and compaction of the villi have gone so far as to have induced substantial or even complete obliteration of the synovial cavity by a mass of brownish yellow spongy tissue. This is what happened in one of our cases. The patient was a young man of 18 who at the time of first admission gave a five and a half year history of difficulty relating to a knee. At this time, the extirpated synovial membrane appeared more or less uniformly bearded and villous. Two and a half years later the patient was admitted because of a recurrence of his difficulties. Now the surgeon found that, though pigmented villous synovial membrane still existed in the intra-articular space, it was continuous with a fist-sized, solid mass of tissue, which he removed from the region representing the quadriceps pouch (fig. 3 *A*). Gross section of this mass showed that no pouch space was left. The tissue filling the space was

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EXPLANATION OF FIGURE 3

A, photograph of a tissue mass (representing a recurrent lesion) removed from a knee joint two and a half years after a pigmented villous synovial membrane had been widely but not completely excised. On the right, the villous character of the intra-articular portion of the synovial membrane is still apparent. The other two thirds of the picture shows, laid open, the mass occupying the quadriceps pouch. The sectioned surface reveals the finely porous character of the tissue, in which the lighter areas represent those more heavily infiltrated with lipoid and the darker those more heavily infiltrated with blood pigment. The patient gave no history of trauma in relation to his knee joint difficulty, which was already of eight years' standing at the the time when this tissue mass was removed. Shortly after this second operation he received a course of roentgen therapy to the affected knee, since there were indications that the lesion might again be recurring. The disorder then quickly subsided, and when the patient was seen recently (almost eleven years after the second operation) there was every indication that the disease had been completely eradicated.

B, photomicrograph (× 12) of a section prepared from the finely porous part of the tissue mass shown in *A*. The narrow branching and anastomosing clefts to be seen are bordered by synovial lining cells. The tissue delimited by these channels is crowded with macrophages containing lipoid or hemosiderin, and also shows some multinuclear giant cells. The areas in which hemosiderin-bearing macrophages predominate appear dark, and those in which lipoid-bearing macrophages predominate appear lighter.

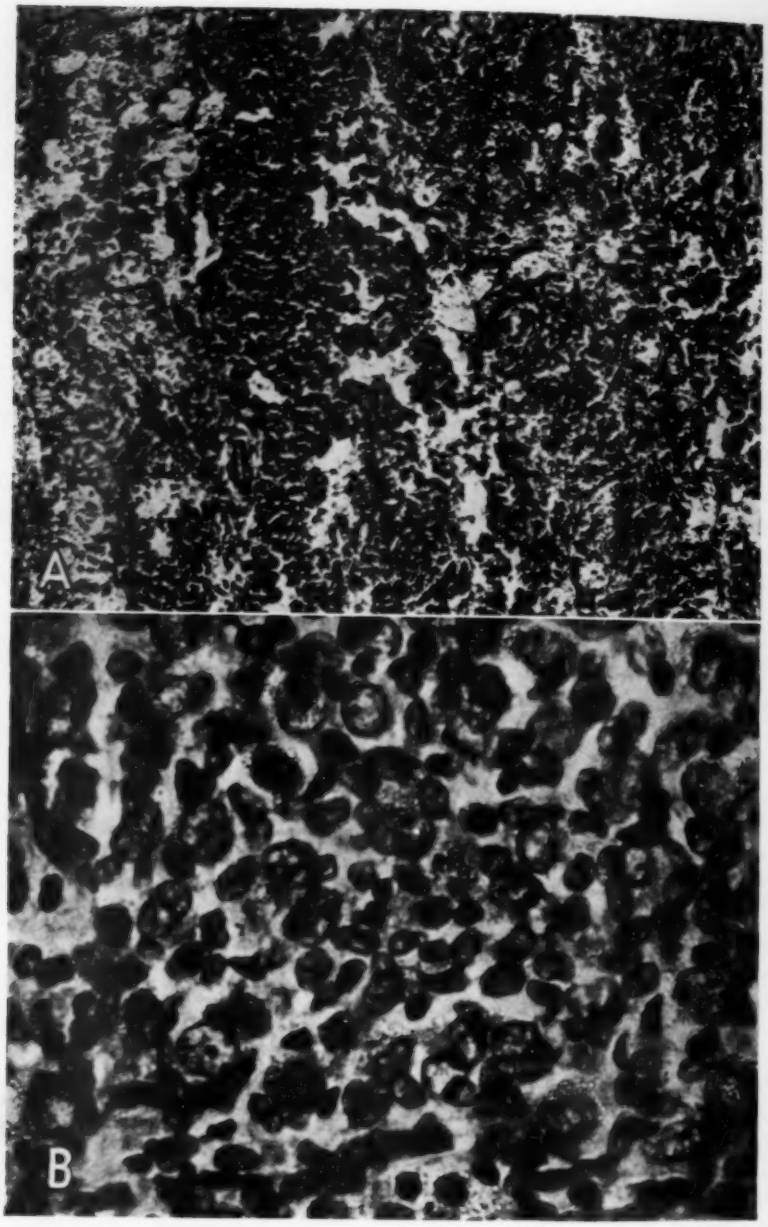

Figure 4

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rather firm in consistency but still distinctly fine meshed except in the firmest parts. Its color ranged from yellowish brown in the firmest areas to dark reddish brown in the more spongy areas, and in some of the spongy parts the color was blackish brown.

Microscopically, extensive and even tumor-like proliferation of the stroma of this agglutinated villous tissue was apparent and indeed dominated the picture. The spaces between the agglutinated villi were found diminished to narrow channels (albeit lined by synovial lining cells), and the pulp of the spongy tissue was correspondingly increased (fig. 3*B*). This pulp presented a highly variegated picture (fig. 4). Freely and richly dispersed, there were closely compacted polyhedral stromal cells without much pigment, polyhedral cells loaded with brown pigment granules, and foam cells with or without pigment. An appreciable number of the polyhedral cells showed mitotic figures reflecting active proliferation of the cells. Variable numbers of multinuclear giant cells were also present, especially in proximity to the nonpigmented polyhedral cells, by whose fusion they had been formed. As noted, the picture was variegated, so that in some places there were whole patches dominated by one type of cell or another, such as foam cells or pigmented or nonpigmented polyhedral cells. Studying many sections, one could gather that in the evolution of this picture the polyhedral cells developed through hyperplasia and modification of the basic stromal cells and only secondarily incorporated pigment or lipoid or both. Actually, the complex appearance of this specimen represented only elaborations and variations of the theme picture presented by the simpler villous lesions.

A plainly villonodular synovial membrane (fig. 5*A*) appears brownishly discolored and studded more or less patchily with individual and clustered nodules, varying rather widely in size. Among the clumps of nodules and elsewhere on the synovial surface one may also see some pigmented villous areas. Some of the nodules are sessile, but most are pedicled, although the pedicle is quite short as a rule. There are usually also some nodules whose appearance indicates quite clearly that the

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EXPLANATION OF FIGURE 4

A, photomicrograph (× 100) of an area from the section shown in figure 3*B*, revealing a region in which lipoid (foam) cells and hemosideric cells are closely intermingled.

B, photomicrograph (× 850) showing the details in an area (from another part of the lesion pictured in figs. 3*B* and 4*A*) consisting mainly of closely compacted polyhedral stromal cells. Many of these cells present fine pigment granules in their cytoplasm. An area like this may show numerous mitotic figures and altogether might not unreasonably suggest that one was dealing with a sarcoma.

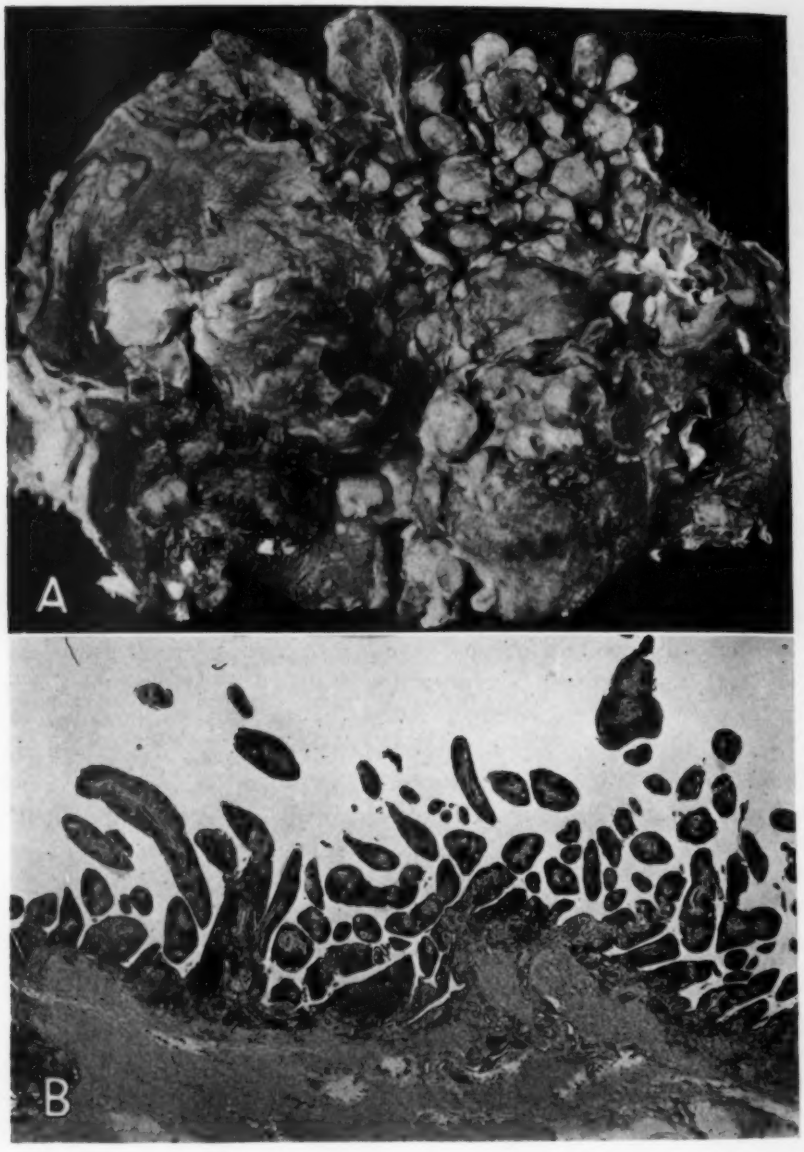

Figure 5

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stalked nodules evolve from sessile ones. In conformity with the latter finding is the observation that on the whole the sessile nodules are much smaller than the stalked ones, many of the former being of only pinhead size. However, a considerable proportion of the nodules—especially the stalked ones—range between 0.5 and 1 cm., and some are even as large as 2 cm. in diameter. The nodular tissue is of an elastic, fleshy consistency. Sectioning of a nodule reveals a smooth surface, yellow-brown as a whole but sometimes streaked with some more pronounced yellow or brown. Here and there on the surface of the synovial membrane one may also find filmlike deposits of fibrin, under which there are usually other residua of hemorrhage.

Microscopically, in a synovial membrane showing mainly simple nodular involvement the more strictly villous portions have essentially the same appearance (except for greater plumpness of the villi) as the diffusely villous synovial membrane (fig. 5*B*). Whether small or large, sessile or stalked, the nodules (fig. 6) in such a synovial membrane present essentially one cytologic pattern. They are lined by one or more layers of more or less pigmented synovial lining cells. The substance of the nodules is composed of compacted masses of large, roundish or polyhedral cells (fig. 7), a number of which in the more cellular areas show mitotic figures. Among these cells there may be a few or even a fair number of multinuclear giant cells. On the whole, the nodules are only moderately vascularized. Especially near their periphery, many of the roundish or polyhedral cells may be packed with granules of brown pigment. Some nodules contain relatively few, while

EXPLANATION OF FIGURE 5

A, photomicrograph of the synovial lining of a knee joint showing diffuse villonodular involvement. One can see both sessile and stalked nodules, and near the lower left hand corner there is a patch which is still distinctly villous. The patient was a woman 31 years of age whose knee joint difficulty was of five years' standing at the time when the synovectomy was done. There was no history of possibly relevant trauma. Evidently the synovectomy alone did not give her permanent relief, since in following up her case we found that three years after leaving our care she sought treatment elsewhere for what was apparently the same condition in the same knee.

B, photomicrograph (× 10) of a section through a villous area of the synovial membrane pictured in *A*. Approximately in the middle of the picture, one can see a dark-staining thick villous protrusion. Under higher magnification the cells composing this are found to be predominantly roundish or polyhedral ones, resembling those shown in figure 7. Similar cells are to be found sparsely distributed in the stroma of the villi, where it is apparent that many are being converted into pigment-bearing phagocytes.

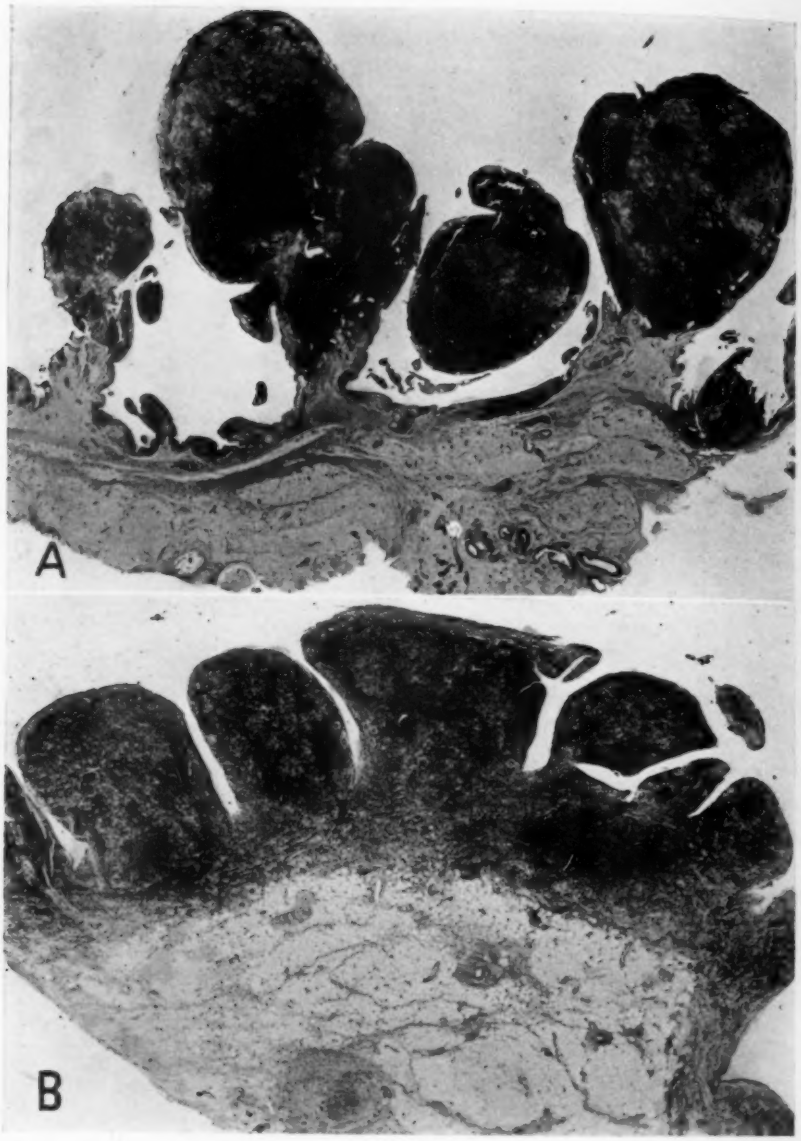

Fig. 6.—*A*, photomicrograph (× 5) of a section through an area showing stalked nodules, from the synovial membrane pictured in figure 5 *A*. Under higher magnification these stalked nodules are found to be very cellular, showing dense collections of polyhedral stromal cells, with intercellular deposits of collagen and hyalin in some places.

B, photomicrograph (× 15) of a section through an area showing sessile nodules, from the synovial membrane pictured in figure 5 *A*. The cellularity (see fig. 7) of the nodules is discernible even at this magnification, the larger black dots representing multinuclear giant cells.

others contain many, scattered clumps of foam cells or lipoid-bearing cells, the major portion of which also show brown granules in their cytoplasm. Practically all of the nodules show some hyalinization of the stroma, this process being manifest around the blood vessels if anywhere. In the larger nodules the hyalinization may be very extensive, hyaline matrix appearing between, and crowding out, the round or polyhedral stromal cells. Sometimes one finds an occasional nodule which has undergone practically complete hyalinization.

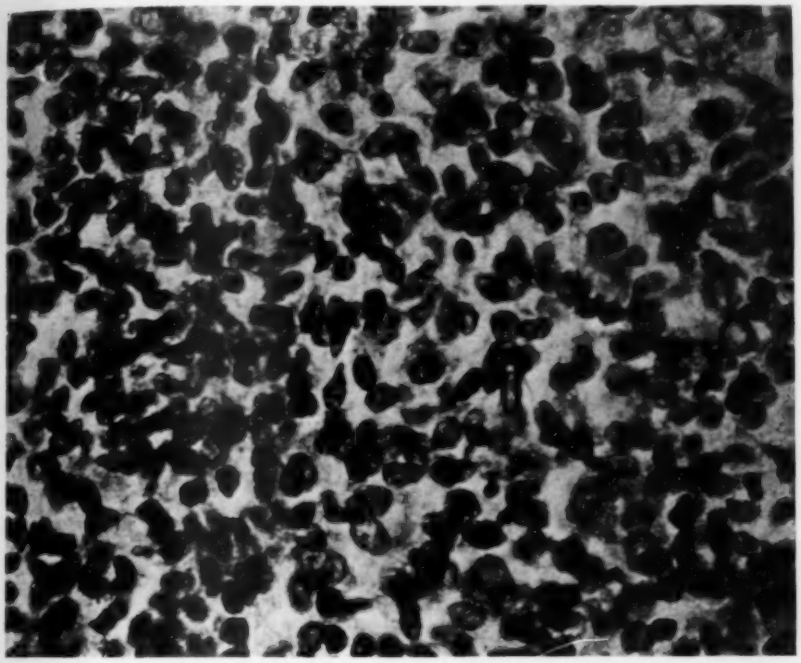

Fig. 7.—Photomicrograph (× 850) showing cellular details in a field selected at random from one of the nodules shown in figure 6*B*. Note the compacted stromal cells, some of which contain granules representing hemosiderin pigment.

A highly complex villonodular lesion (fig. 8), created by intergrowth and fusion of the villous and nodular masses, may also sometimes be found. Such an elaborate picture was presented, for instance, by one of our specimens, coming from a woman of 46 with a history of knee joint difficulty of about five years' duration. The intra-articular portion of this synovial membrane presented a brownish villous and nodular appearance. The quadriceps pouch, however, was represented by a fist-like mass of tissue. When this was cut open, it was found that the

pouch space was represented by several small compartments, between which were thick septums of tissue. In several of the compartments the pathologic findings included large pedicled nodules still attached to the walls. The dividing septal walls, though rather fibrous, still revealed, here and there, indications that they had been formed through intergrowth of nodular and villous tissue. Specifically, one could find, along with some brownish spongy tissue, also individual nodules at various stages of agglutination and fusion.

On the whole, the cytologic appearance of the individual nodules in these more exuberant lesions (fig. 9*A*) is basically similar to that in the simpler villonodular lesions. On the other hand, the tissue repre-

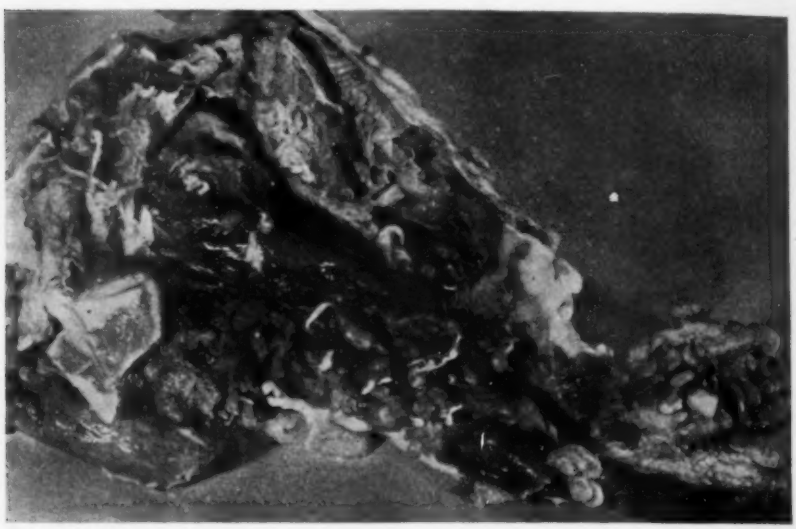

Fig. 8.—Photograph of the synovial membrane of a knee joint representing a complex villonodular lesion. On the right the stalked nodular and villous character of the intra-articular portion of the synovial membrane is still apparent. The other two thirds of the picture shows, laid open, the mass which occupied the quadriceps pouch in the case in question. The sectioned surface reveals, though not too clearly, irregular compartments with thick tissue septums between them. The patient was a woman of 46 with a history of knee joint difficulty of at least five years' standing. There was no history of possibly relevant trauma. When the case was followed up recently (three years after the synovectomy) the knee was still creating difficulty and presented indications that the lesion had recurred. This is another of our cases showing that synovectomy is not likely to eradicate the disease unless this procedure is followed by radiation therapy.

sented by the agglutinated and intergrown nodules and villi is of a richly cellular character. Indeed, its microscopic appearance can be

bewildering at first sight. One sees smaller or larger cleftlike spaces lined by synovial cells and bordered by wide stretches of tissue the component cells of which are spindle shaped, roundish or polyhedral (fig. 9*B*). These cells are often collected into nodular agglomerations, and many may contain pigment granules. They are interspersed by variable numbers of multinuclear giant cells and lipoid-containing foam cells. Altogether, such complex specimens may be regarded as representing the nodular equivalent of the closely matted villous lesion previously described, in which the quadriceps pouch was occupied by a mass of spongy tissue.

Circumscribed Form.—The limited extent of the involvement in this form is notable. Most often, the lesion consists essentially of a single nodular outgrowth (fig. 10*A*) from somewhere in the synovial membrane. The nodule may be sessile but is more likely to be stalked. Its knob may measure from less than 1 cm. to several centimeters in its largest diameter. The knob may be roundish or discoid, smooth or even lobulated. The stalk may be quite short or as much as several centimeters in length. The basic color of the knob, and often of the stalk too, is yellow or yellow-brown. In consistency, the nodule is firm and elastic on the whole. The synovial membrane immediately around the stalked or sessile nodule is likely to be but little altered, but it may show slight brownish pigmentation and even slight villous transformation. In a knee, the single nodule frequently issues from the synovial membrane at a meniscocapsular junction. On the other hand, it may be situated in the synovial membrane at the intercondyloid fossa or eminence, near the patella or, less frequently, at some still different site.

Some notice should also be taken of cases in which two or even three stalked or sessile nodules have been found (fig. 10*B*). In regard to such cases, it is sometimes difficult to decide whether they should still be held to represent the circumscribed form or interpreted as representing an early stage of the diffuse form of pigmented villonodular synovitis.

At any rate, the cytology of an individual nodule in this circumscribed form of the condition is basically that of the nodules in the diffuse form of involvement (fig. 10*D*). Furthermore, it also corresponds rather closely to the cytology of the tendon sheath lesion commonly designated as xanthoma or giant cell tumor of the tendon sheath. The surface of the nodule is covered by one or more layers of synovial lining cells, and many of the latter may be filled with granules of hemosiderin. The substance of the nodule is likely to be quite cellular. When it is, one finds it composed of aggregated or diffusely distributed cells, which in general are roundish or polyhedral but here and there (especially

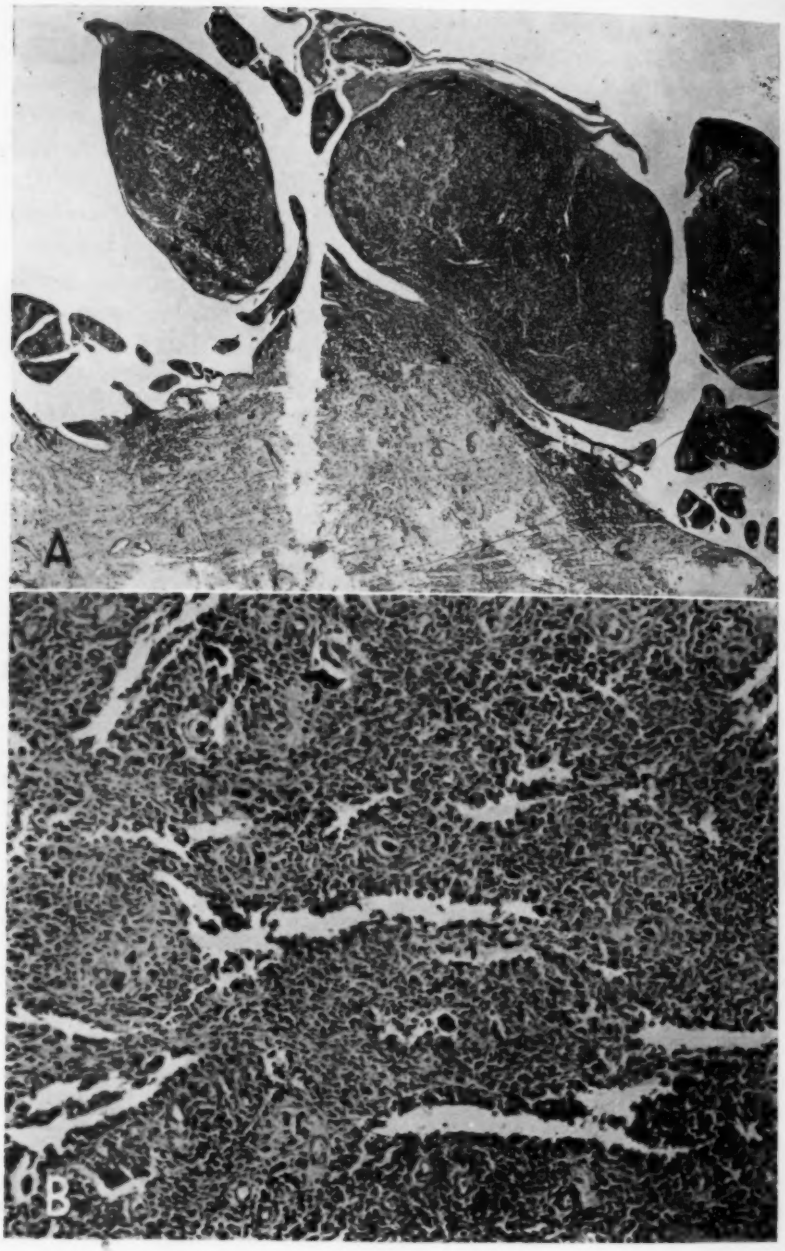

Figure 9

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where collagenization or hyalinization is taking place) are spindle shaped. It is not difficult, in examining fields of these cells, to find some presenting mitotic figures. An appreciable number of the cells may be found packed with hemosiderin pigment. Others may be seen to have taken up lipoid or to have been converted into foam cells. Indeed, some of the lipoid-bearing cells also contain brown pigment. Scattered among the roundish or polyhedral stromal cells are variable numbers of multinuclear giant cells. The latter may also show phagocytic qualities, since some of them contain not only granules of hemosiderin but also engulfed pigment-bearing macrophages. The less cellular lesions are the ones in which collagenization and hyalinization are more pronounced, and in these one may find large patches of poorly cellular connective tissue.

Not infrequently, a stalked outgrowth of the synovial membrane is encountered which is more or less fibrotic and necrotic and whose center is rather rich in cholesterol crystals. The genesis of such lesions is not always entirely clear. Certain of them, however, indubitably represent lesions belonging originally to the type just described but modified by crushing in the course of articular function and by the secondary effects of such crushing.

CLINICAL ASPECTS OF THE SYNOVIAL INVOLVEMENT

Diffuse Form.—The patients who show the diffuse form of synovial involvement are nearly always young adults. Indeed, the ages of our 11 patients presenting such involvement were as follows: 18, 20, 22, 22, 23, 23, 27, 31, 36, 46, 48. The lesion is perhaps more common in males than in females. Specifically, 8 of our patients were males and only 3 were females, but the total number is obviously too small to permit definite conclusions on this point.

The interval between the appearance of clinical difficulties and medical consultation was only a month in 1 case and as much as five years in another, though the average interval for the 11 cases was two to three

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EXPLANATION OF FIGURE 9

A, photomicrograph (× 20) of a section through an area showing stalked nodules and some villi from the synovial membrane pictured in figure 8. The general cellularity of the nodules is discernible even at this magnification, and one can also see how the dark spots representing multinuclear giant cells are scattered through the nodules.

B, photomicrograph (× 100) of a section prepared from the wall of one of the compartments occupying the quadriceps pouch in the specimen shown in figure 8. Note the cleftlike spaces delimited by synovial lining cells and the mixed character of the stroma between these clefts.

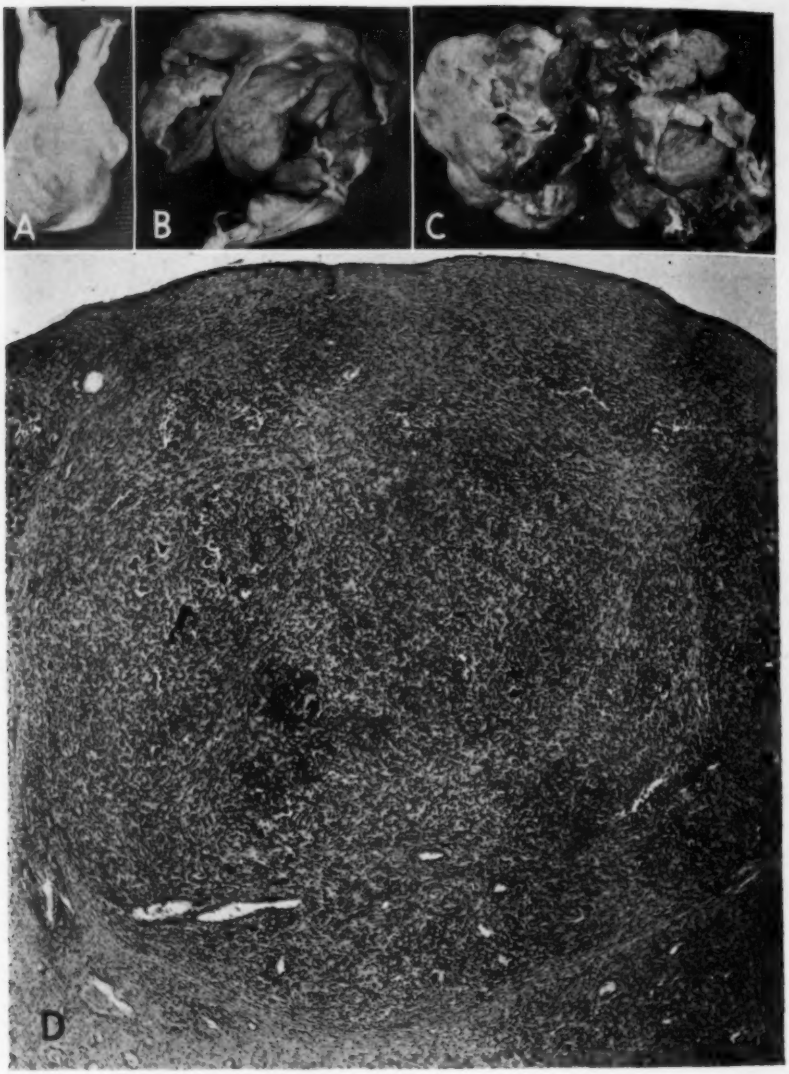

Figure 10

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years. The important complaints are pain and swelling in the affected joint—a knee in all our cases except one. These complaints are often intermittent. Less common difficulties are stiffness on flexion, locking, snapping on sudden extension, and limp. A history of trauma was elicited in only 3 of our 11 cases, and this hardly seems to bear any causal relation to the condition.

As to diagnosis, roentgenography is of little value except so far as it confirms the diagnosis of fluid in the joint and swelling of the soft tissues. The various clinical laboratory tests are of no particular value here. It is true that the literature contains references to the presence of hypercholesteremia. However, in those 4 among our cases in which the cholesterol values were determined, these values were found within the upper limits of the normal range, the total cholesterol being 150, 177, 200 and 210 mg. per hundred cubic centimeters, respectively. Furthermore, the total cholesterol–cholesterol ester ratio was also within the normal range.

A finding which does help in the diagnosis, and which may indeed by itself suggest the condition, is the aspiration of serosanguineous fluid from the knee. In a case in which the diagnosis is plausible on other scores also (e. g., that of a young subject known not to be a hemophiliac and giving no history of recent severe trauma to the knee) this finding, especially the repeated aspiration of serosanguineous fluid from the knee, strongly indicates the presence of pigmented villonodular synovitis. (Indeed, when the surgeon opens the joint, he usually encounters cloudy brownish or even dark or reddish brown fluid. This may contain clots of fibrin, and fibrin may also be found deposited in the articular recesses.)

EXPLANATION OF FIGURE 10

A, photograph of a solitary stalked lesion from the knee joint. It was removed from the synovial membrane a little above the internal meniscus. The paleness is due to the fact that the nodule is substantially necrotic as a result of having been crushed in the course of articular function. The patient was a girl of 18 whose difficulty, starting without any antecedent trauma, dated back only one month before the operation.

B, photograph of a circumscribed but multinodular lesion of the synovial membrane of a knee. The site of the lesion was not far from the internal meniscus. The patient was a woman of 38 whose difficulty began at least two and a half years before examination and who related it to a fall that occurred about three years before examination. No follow-up history was available in this case.

C, photomicrograph of a partially opened pigmented villonodular bursa, removed from around the ankle region. One can make out the villous nature of the lining in various places, and a clearcut nodule is rather plainly visible in the center of the picture.

D, photomicrograph (× 30) of a section through one of the nodules from *B*, showing the general cytologic pattern of such a nodule. Compare with figure 9 *A*, for instance.

Circumscribed Form.—Our patients with this type of synovial involvement were considerably older, on the average, than those with the diffuse form. This trend in regard to age incidence is confirmed, on the whole, by the pertinent data in the articles by Harbitz [8] and De Santo and Wilson.[6] In our 9 cases the ages were as follows: 18, 18, 37, 38, 40, 43, 48, 52, 68. As to sex incidence, there seems to be no predilection for one sex or the other. Though it was almost always a knee joint that was affected, we did have a case in which the lesion was in an ankle joint.

The average duration of the clinical difficulties to the time when the patients came under observation was four and a half (4.5) years, though in 1 case the symptoms dated back as short a time as one month and in another as long as eight years. On the whole, the difficulties are mild—a fact which in itself explains why these patients do not usually seek medical advice early. Not infrequently, there is a history of swelling of the affected joint, relatively slight in amount and intermittent in character. The swelling is usually due to effusion, but the latter is rarely abundant enough really to require aspiration. However, if this is done, it yields a straw-colored fluid, which is found sterile when cultured. Pain, often intermittent like the swelling, is also not infrequently reported. Other complaints which may be noted when the knee is affected are episodes of locking and some limitation of motion (particularly extension). A history of an acute local trauma (which may date back many years) was obtained in only a few of our cases, and since it was more often absent than present it probably has no causal significance.

Physical examination may confirm the impression of slight swelling or enlargement of the joint. The examiner not infrequently senses, furthermore, that the joint contains a movable mass, which explains why in so many of these cases the trend of the preoperative diagnoses is toward "joint mouse," osteochondritis dissecans, dislocated or torn meniscus, and so on. Roentgenography is of no particular help in diagnosis. In fact, the correct diagnosis is usually not made until the joint is opened and the lesion exposed and examined.

PIGMENTED VILLONODULAR BURSITIS

As previously indicated, the diffuse lesion of the synovial membrane has its exact counterpart in bursae mucosae. However, such a lesion is not commonly encountered in bursae mucosae. Our own records include 2 instances of involvement of a popliteal bursa (without concomitant involvement of the knee joint), 1 of involvement of the bursa anserina and 1 of involvement of an ankle bursa. The ages of the subjects

8. Harbitz, F.: Arch. Path. **4**:507, 1927.

were 28, 28, 34 and 42 years. Three were females. All had noted for at least three years, and 1 patient had noted for seven years, the presence of a mass at the site affected. It was clearly stated by 2 patients that they knew of no relevant trauma, while the histories of the other 2 contain no information on this point. Some had noticed that lately the mass had been slowly enlarging and that it was somewhat painful. In some cases it was rather tender to palpation, but in none had it interfered significantly with articular function. In none of the cases was a precise diagnosis of the condition made on a clinical basis.

The extirpated bursa was at least 5 or 6 cm. in its greatest diameter. The bursal cavity was still present in some instances and more or less obliterated in others. When it was retained, its wall was 1 cm. or more in thickness and showed a lining of brownish discolored villous fringes and often also many polypoid projections (fig. 10 *C*). When it was more or less obliterated (through fusion of the villous and nodular tissue growing inward from the wall), the rather solid lesion was of spongy consistency and also had a brownish pigment. In such a case it was sometimes even difficult to be sure that the structure in which the condition developed was actually a bursa. Not only in gross appearance but also microscopically (fig. 11 *A*) these diffuse lesions of bursae mucosae resemble the diffuse ones of the synovial membranes, and what has been said of the microscopic anatomy of the latter applies equally well to the former.

PIGMENTED NODULAR (OR VILLONODULAR) TENOSYNOVITIS

The first step toward the more modern conception of the so-called xanthoma or giant cell tumor of the tendon sheath was taken by Heurtaux [9] in 1891. He appreciated the special character of the lesion in question and stressed the idea that it was not a sarcoma as previous writers had thought. Since then there has accumulated a large body of literature on the condition under consideration. Among the more recent reviews of the subject are those by Lecène and Moulonguet,[10] Mason and Woolston,[11] von Albertini,[12] King,[13] Geschickter and Copeland [14] and Galloway, Broders and Ghormley.[7]

Incidence, Localization and Clinical Aspects.—Galloway, Broders and Ghormley [7] indicate that the literature to 1940 records 274 cases

9. Heurtaux, A.: Arch. gén. de méd. **27**:40 and 160, 1891.

10. Lecène, P., and Moulonguet, P.: Ann. d'anat. path. **1**:393, 1924.

11. Mason, M. L., and Woolston, W. H.: Arch. Surg. **15**:499, 1927.

12. von Albertini, A.: Gutartige Riesenzellgeschwülste, Leipzig, Georg Thieme, 1928.

13. King, E. S. J.: Brit. J. Surg. **18**:594, 1931.

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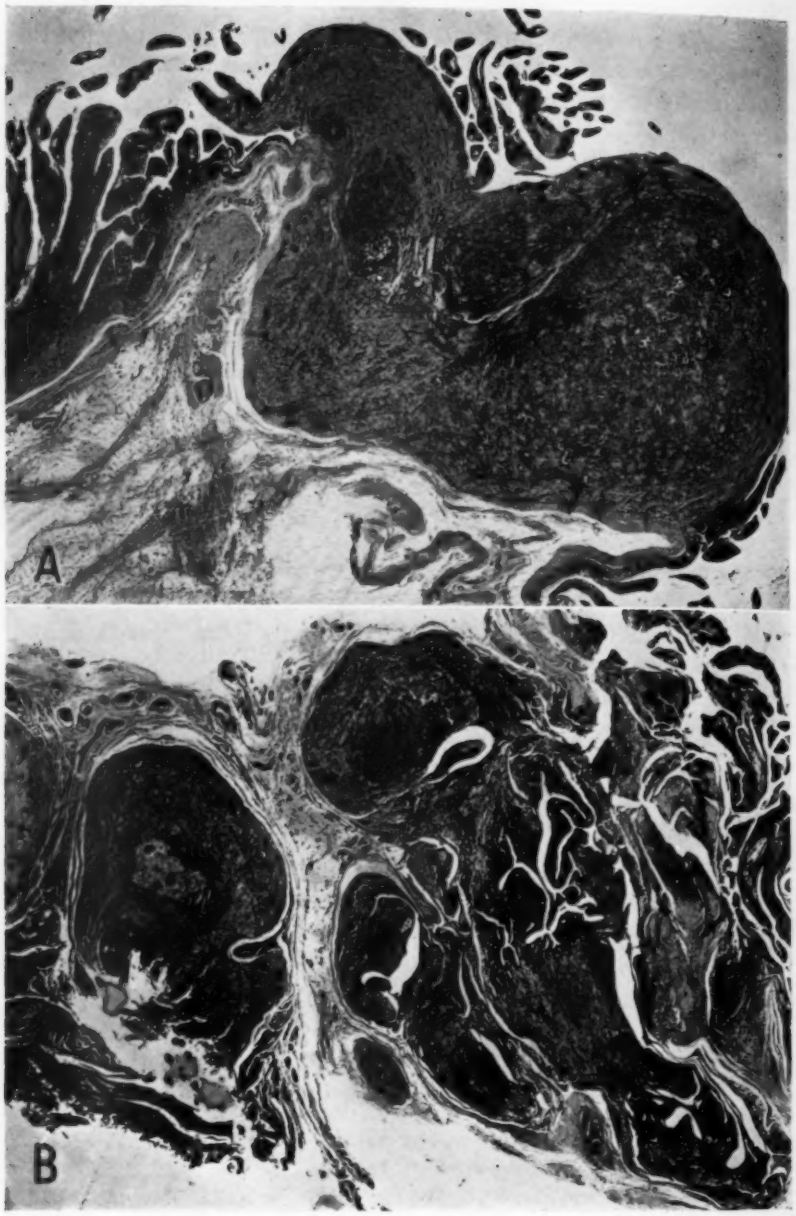

Figure 11

(See legend on opposite page)

of the so-called xanthoma or giant cell tumor of the tendon sheath, apart from their own cases. However, the condition is certainly more common than this figure suggests. For example, they themselves report 65 cases, and our own experience includes 55 hitherto unreported cases. In the large majority of all the instances the disorder occurred somewhere between the wrist and the finger tips, and in almost all the rest, between the ankle and the toe tips. Within this range, it was found far more often in fingers than anywhere else. Characteristically, also, there was but a single lesion, usually from 1 to 3 cm. in its greatest diameter and restricted to one sheath. Some of the reports indicate that occasionally there are two (or even more) distinct lesions within the same sheath and that more rarely several tendon sheaths show individual lesions. As to the fingers, there seems to be no particular predilection for any one digit. Within a given digit, the lesion was found more often toward the proximal than the distal end, and indeed it sometimes extended on to the palm or the back of the hand. Furthermore, the flexor was affected far more often than the extensor sheath.

EXPLANATION OF FIGURE 11

A, photomicrograph (× 11) of a section through an area of the bursal wall shown in figure 10*C*. On the right there is a large cellular nodule, bordered in some places by villi. On higher magnification this nodule is seen to contain foam cells, lipoid-bearing phagocytes and multinuclear giant cells and to be undergoing hyalinization. To the left there are plump, dark-staining villi which on higher magnification are seen to be very rich in pigment-bearing phagocytes.

B, photomicrograph (× 11) demonstrating various stages in the evolution of pigmented villonodular tenosynovitis toward the compact tumor-like tendon sheath lesion which usually goes under the name of xanthoma, giant cell tumor, and so on. At the extreme right one can see dark villi which under higher magnification are seen to be crowded with hemosiderin-bearing phagocytes. Somewhat to the left of these villi one can note sinuously winding channels where villi have become invaginated and agglutinated. The two cellular nodules—one at the upper center and one toward the left in the picture—represent still more advanced evolutionary stages proceeding from obliteration of the spaces in the course of stromal cell proliferation. Again, under higher magnification these nodules are seen to be composed of compacted agglomerations of foam cells and hemosiderin-bearing phagocytes, with some multinuclear giant cells strewn among them. At the extreme left of the picture one can see a part of such a nodule, which has undergone still further evolution and become almost completely hyalinized. The specimen comes from the sheath of the flexor pollicis longus muscle and specifically from a site near the metacarpophalangeal joint. The patient, a woman 35 years of age, stated that she had noted the presence of the "tumor" on her left thumb for only two weeks prior to admission to the hospital and had not related its appearance to any trauma. The likelihood is that the lesion was of more than two weeks' standing; nevertheless it is precisely because of its youth that it still showed early stages of evolution along with the later ones, familiar from most of the relevant tendon sheath lesions.

The condition seems to show a predilection for females. The persons in whom it is observed are usually young or middle-aged adults. The subjects often report merely that they have a nodule (on a finger or a toe, or around a wrist or an ankle) which, after growing slowly for years, has lately begun to grow rapidly. Only occasionally do they complain of pain or interference with motion. These complaints arise when the lesion is impinging on a nerve or on the underlying tendon or has become particularly large. The skin over the lesion is usually movable. The correct diagnosis can frequently be established preoperatively if one bears in mind that a small nodular mass of fibrous consistency situated on the fingers is in all probability a lesion of this character.

Sometimes, on a toe or a finger the lesion attains a diameter of 5 cm. or more and even erodes the neighboring bones. Thus in a case in our series it had infiltrated the fascial sheaths and tendons of a toe and had substantially destroyed the two proximal phalanges. Such conditions are exceptional but have also been noted by others—for instance, by Harbitz,[8] in relation to a finger. When the condition appears in a wrist or an ankle region, it also not unusually attains considerable size and is no longer restricted to a single sheath. Mason and Woolston[11] and Lecène and Moulonguet[10] have reported pertinent cases in which the wrist and ankle regions, respectively, were involved.

The literature contains statements to the effect that the tendon sheath lesion in question may be associated with hypercholesteremia or at least some disturbance in the total cholesterol–cholesterol ester ratio. In none of our cases studied from this point of view was there any alteration in the blood cholesterol values, and we wonder whether reported cases in which such alteration was found do not actually represent instances of xanthoma tuberosum multiplex.

Pathologic Features.—At operation, if the lesion is a small solitary one, it usually, despite its location in the tendon sheath, has not invaded or narrowed the space between the sheath and the tendon. Typically, such an extirpated lesion is not more than a few centimeters in its greatest diameter and of a rather firm fibrous consistency. It is likely to be somewhat flat from compression, and its external surface is usually lobular. When it is sectioned, its cut surface, too, often presents a somewhat lobulated appearance. Its color may range from whitish gray to yellowish or reddish brown. In a particular specimen, the coloring is usually mottled rather than uniform throughout. The intensity of the yellow and brown discoloration reflects in a general way the abundance of lipoid and hemosiderin, respectively.

On microscopic examination, the "tumor" is seen to be invested by a thin fibrous capsule from which septal strands extend inward. Though all the lesions show a basically similar cytologic pattern, which may

be recognized at a glance, the details of this may vary considerably from lesion to lesion and even within the several lobules of the same lesion. In a general way, these variations reflect the progress of the lesion from an initial villous pigmented aspect, through an intermediate nodular and cellular phase, to a rather meagerly cellular, fibrous and hyalinized end stage. The whole pattern of development can be worked out only if many individual specimens are studied and arranged with the object of ascertaining the evolutionary sequence of events. If this is done, one occasionally encounters, as already noted, a lesion (figs. 11 *B*, 12 *A* and *B*) which on microscopic examination shows not only the usual solid, more or less cellular lobules undergoing fibrous obliteration but also, in some areas at least, the earlier transient villous pigmented stage. The reason why one does not often encounter a lesion still showing the earlier evolutionary phases is that most of the tendon sheath "tumors" develop so insidiously and give rise to so few troublesome complaints while they are still small that they have generally attained an advanced stage of development and indeed even have undergone fibrous involution by the time they are extirpated.

Where it is still villous in character, the tendon sheath lesion is highly vascularized, and perivascular blood extravasations may be observed in the sublining tissue. Furthermore, the villi all contain more or less brownish pigment. Where they have coalesced, small nodules are observed, often incorporating clefts delimited by several layers of hypertrophied and pigmented tenosynovial cells. Some of these nodules may become impregnated with lipoid, but by no means all. Those which are free of lipoid may be quite cellular. The cells are for the most part large polyhedral ones containing roundish or vesicular nuclei, and, as previously indicated (in connection with the lesions of the synovial membrane and the lining of the bursa mucosa also), it is these multipotent cells which apparently give rise not only to the hemosiderin-containing macrophages and the lipophages but also to the multinuclear (giant) cells within the lesion. In richly cellular areas the intercellular matrix may be relatively meager. On the other hand, the intercellular material may be abundant in some places or throughout the lesion, and it is then either distinctly fibrillar or hyaline. Indeed, even in highly cellular areas, one may see beginning foci of fibrosis and hyalinization, which coalesce and become larger until eventually the entire lobule is scarred. The cells are apparently crowded out in this process, and in the acellular hyalinized end stage of the lesion there remain only scattered small cleftlike or roundish spaces bordered by synovial lining cells. Small multinuclear (giant) cells may be prominent in relation to such spaces, which may furthermore contain nests of hemosiderin-laden macrophages or of lipophages. Moreover, cholesterol crystals are sometimes observed in such fibrous nodules, in addition to the more common "foam" cells.

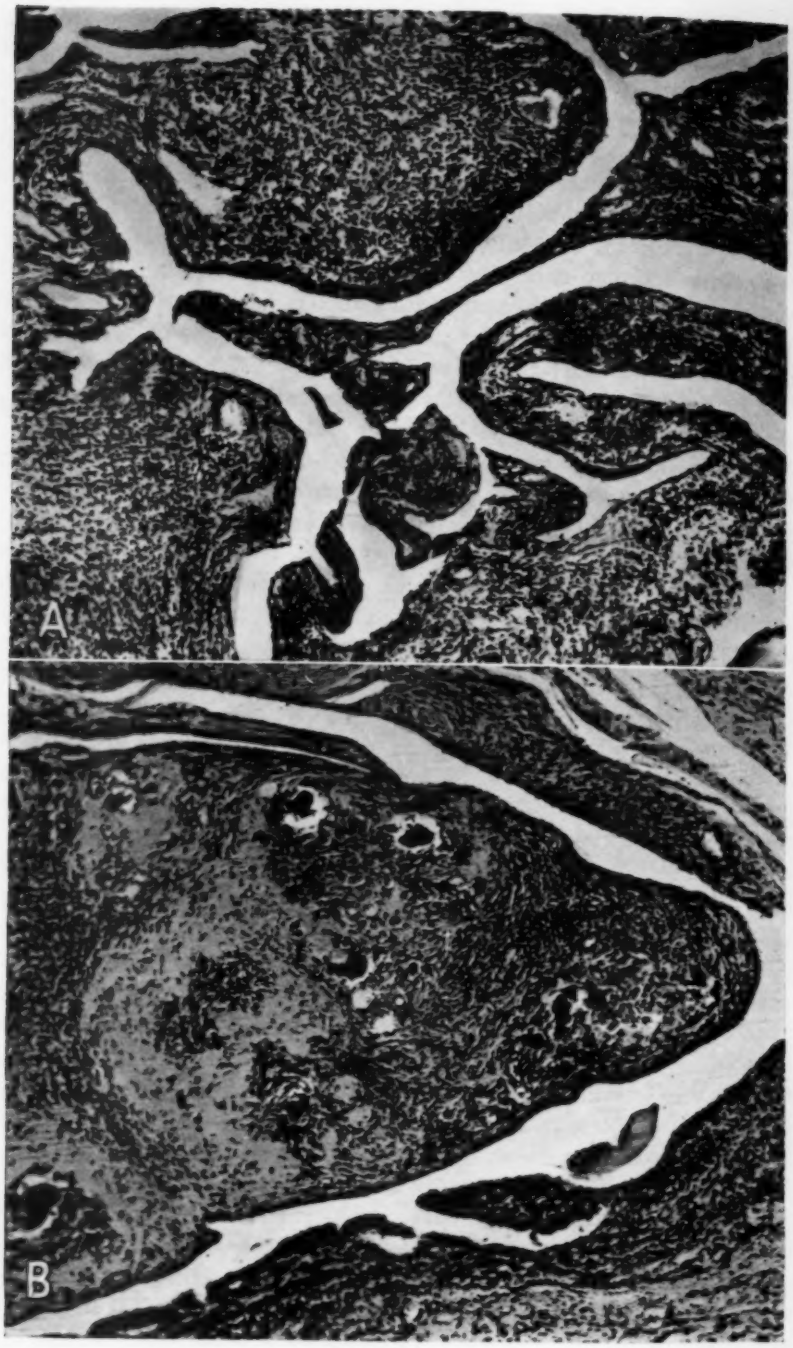

Figure 12

(See legend on opposite page)

In regard to the extent of lipoid deposition, the individual lesions or different parts of them vary widely. Thus one may find abundant aggregates of foam cells in one or more lobules of the lesion, and even note that many of the stromal cells have lipoid in them; on the other hand, one may actually have to search for foam cells, though if one stains sections for fat, the stromal cells may nevertheless show lipoid granules. The lipoid in these various cells is mainly cholesterol esters. In an occasional lesion (especially, it seems, a large one that has been subjected to trauma) small necrotic areas may be seen, containing cholesterol crystals. The giant cells, apparently formed by fusion of the stromal cells, may also be numerous or sparse, scattered or collected in foci. In any event, the giant cells represent a distinctive feature of the lesion, even though they do not necessarily dominate its cellular makeup.

The hemosiderin content of individual tendon sheath lesions is likewise somewhat variable. Some lesions present abundant hemosiderin; others contain comparatively little and show focal distribution of whatever pigment there is. The hemosiderin may be extracellular but is more often deposited in the spindle-shaped or polyhedral stromal cells.

Altogether, these lesions of tendon sheaths present striking histologic similarities to the analogous lesions to be found in the linings of joints and bursae mucosae. However, the tendon sheath "tumors" on the whole are less vascular and show a more pronounced tendency toward fibrosis and hyalinization of the intercellular matrix. Furthermore, they are usually somewhat richer in lipoid but less strongly pigmented by hemosiderin. In these respects they resemble the localized stalked lesions of the synovial membrane more closely than they do the diffuse lesions of joints or bursae. We have mentioned the occasional instances of diffuse involvement of many neighboring tendon sheaths (for instance, around a wrist or an ankle) and other exuberant tendon sheath lesions such as sometimes lead to pressure erosion of the bones of fingers or toes. These diffuse tendon sheath lesions may be regarded as the anatomic analogue of the diffuse lesions of the synovial membranes in which the villi and nodules have become matted together into a large tumor-like mass which, in the cases in which the knee joint is involved, occupies the whole quadriceps pouch.

EXPLANATION OF FIGURE 12

A, photomicrograph (× 75) showing in greater detail the area with the sinuously winding channels mentioned in connection with figure 11 *B*. These channels are lined by pigmented and hypertrophied synovial lining cells, which border the agglutinated and hypertrophied villous tissue.

B, photomicrograph (× 100) showing an invaginated villous fold from the specimen of figure 11 *B*. This fold already shows hyalinization of the stromal tissue and multinuclear giant cells in the more cellular areas. It is appearances like these that account for the common designation "giant cell tumor."

COMMENT

The various anatomic expressions of the lesion which we are calling pigmented villonodular synovitis have been described. Within the total framework, we indicated the place of the circumscribed form of the lesion, consisting of a solitary stalked or sessile synovial outgrowth (or at most a few such outgrowths) and often named in the past xanthoma, giant cell tumor or xanthomatous giant cell tumor of the synovial membrane. Notice was also taken, as it has been by others, of the close cytologic correspondence of such an outgrowth of the synovial membrane to the equivalently named lesion of the tendon sheath. Indeed, it was found possible to show that the tenosynovial and synovial lesions in question not only correspond cytologically in their fully developed form but also go through essentially the same sequence of evolutionary stages in reaching this form. This is why the tenosynovial lesion of the type commonly denoted as xanthoma, giant cell tumor, xanthogranuloma, and so on, has been drawn within the scope of this paper, under the head of pigmented nodular (or villonodular) tenosynovitis.

Attention was also devoted to the diffuse pigmented villonodular synovial lesion and to the cytologic and developmental similarities of individual areas of this to the solitary stalked synovial and even the tenosynovial lesion. How complex a diffuse lesion of a synovial membrane can become through matting together of villi and nodules was a point also stressed, but in following the genetic sequence of events in various parts of such a complex lesion it was made plain how it is linked by traceable evolutionary stages to the simpler lesions. Thus these complex lesions have been interpreted as belonging within the framework of pigmented villonodular synovitis, and it was pointed out that they should not be classified, as they have sometimes been, under such heads as giant cell fibrohemangioma, fibrohemosideric sarcoma, sarcoma fusogigantocellulare and benign or malignant polymorphocellular tumor of the synovial membrane. Finally, some attention has been devoted also to pigmented villonodular bursitis as an almost perfect analogue of villonodular synovitis.

As to cell composition, emphasis was laid on the strategic polyhedral stromal cells to be found strewn in the villi or agglomerated in the nodules of the synovial, tenosynovial and bursal lesions alike. In particular, it was pointed out that these stromal cells are potential phagocytes and that indeed, soon after they make their appearance, many of them contain granules of hemosiderin. It was also noted that subsequently these cells exhibited additional phagocytic qualities in that many of them take up lipoid (becoming converted into foam cells) and that through fusion they may form multinuclear giant cells, which are also to be regarded as phagocytes.

The question of the causation and nature of the condition is a controversial one. Is the lesion in its various sites and forms a true tumor in an oncologic sense? Many writers maintain that it is, at least by implication in the use (based on certain cytologic characteristics) of such names for it as "xanthoma," "giant cell tumor," "fibrohemosideric sarcoma" and "polymorphocellular tumor." These names spring from the presence of, and relative importance attached to, the xanthic (foam) cells, multinuclear giant cells and hemosideric cells, or (in the case of "polymorphocellular tumor") all of them together. As has been pointed out, however, these various types of cells have a common origin in cells derived from the basic connective tissue cells of the affected structure's lining membrane. Specifically, primitive, undifferentiated connective tissue cells in the membrane proliferate to form large numbers of roundish or polyhedral stromal cells which, in turn, give rise to the foam cells, hemosideric cells and multinuclear giant cells. However, in an early or, on the other hand, sometimes in a late stage of evolution of the lesion one may have to search many fields to find a foam cell or a giant cell. This fact, coupled with the fact that the very cells around which the conception of the lesion as a tumor is built are potential or actual macrophages, argues strongly against the supposition that the lesion is a true tumor in the oncologic sense.

A wholly untenable interpretation of the lesion as a tumor is the one proposed by Geschickter and Copeland.[14] They maintained that the condition in question—particularly as appearing in tendon sheaths—develops through tumorous proliferation of osteoclasts in sesamoid bones. All the facts point away from this contention. Thus, in relation to tendons, the "tumors" are in the sheaths, rather than in the tendons themselves, where sesamoids are located. Also, the lesions often occur in the sheaths on the back of the hand, on the dorsum of the foot and around the wrist and ankle, where not even the tendons have sesamoid bones. Finally, the sesamoid osteoclast theory offers no explanation whatever for the occurrence of analogous lesions in articular capsules and bursae mucosae.

Does the condition represent an inflammatory reaction of the affected tissue to irritation from repeated local hemorrhage? As to this question, it is true that the presence of hemosiderin in the superficial and subsurface cells of an affected synovial membrane, for instance, is to be noted at an early stage in the development of the lesion. However, there is nothing to indicate that hemorrhage had occurred before the lesion was already partly evolved. Assuming even that this hemosiderosis is due to absorption of blood into the synovial membrane, following on compression and laceration of vascular villi in the course of articular function, the intra-articular hemorrhage cannot account for the other cytologic features of the fully developed lesion. In this connection, the

findings in affected joints in a case of hemophilia which we had the opportunity of examining are illuminating.[15] In this case, repeated intra-articular hemorrhage had led to pronounced hemosideric pigmentation of the affected synovial membranes, which also became thoroughly villous, matted and hypervascular. However, nowhere in any of the joints did a synovial membrane present the other cytologic features of pigmented villonodular synovitis—foam cells and multinuclear giant cells among nodules or diffuse sheets of hyperplastic stromal cells.

As additional evidence against the factor of repeated intra-articular hemorrhage per se as giving rise to the picture of pigmented villonodular synovitis, we offer, for what they may be worth, certain experimental findings. Specifically, we found that the repeated injection of autogenous blood into the knee joints of rabbits induced but little permanent change in the synovial membranes. When ferric salts were injected—e. g., ferric chloride in aqueous solution—a rather pronounced inflammatory fibrosis of the sublining tissue was obtained, in addition to fixation of the iron pigment within phagocytes in the superficial and subsurface cells of the synovial lining. However, ferric chloride is known to be irritating to tissue, so that the inflammatory response which it provokes can have no special significance here in view of the absence of any pronounced or lasting reaction to autogenous blood.

The consideration of hemorrhage also raises the question of trauma. It seems clear that no crucial significance is to be attached to the latter in the causation of the condition. As noted, some subjects do give a history of trauma in connection with hemorrhage, but most do not. Biebl [16] described 2 examples of a condition which he calls "lipophagic synovitis" and "lipophagic granuloma," limited to a part of the joint and sometimes developing in joints after severe trauma with intra-articular fracture. In its microscopic anatomy this lesion does not resemble the one under discussion. Instead, it is apparently the intra-articular equivalent of the traumatic fat necrosis that is more familiar in relation to the breast or to subcutaneous tissues elsewhere.

Another theory is that the lesion under discussion represents merely an inflammatory response to an imbalance (especially local) of cholesterol metabolism. Support of this idea has been offered by various writers in blood cholesterol figures purporting to show the presence of hypercholesteremia in some cases. However, in none of our cases in which the blood cholesterol was determined was there hypercholesteremia. It is true that the lipoid which accumulates in these lesions is of the nature of cholesterol and that in the florid stage the lesion may contain

15. The source of the material on which our study of this case was based was the personal museum collection of the late Jakob Erdheim, pathologist, of Vienna —a collection now housed in the laboratory of the Hospital for Joint Diseases.

16. Biebl, M.: Arch. f. klin. Chir. **191**:237, 1938.

much of this lipoid. Nevertheless, though these lesions are lipophilic, the concentration of cholesterol in them does not indicate that local cholesterol imbalance is necessarily the causative agent. Indeed, it is not unusual to find lipoid impregnation of a malignant tumor—even one that is not a hypernephroma, for instance—and certainly no one would argue that such a tumor represented a response to cholesterol imbalance. It is also known that lipoid (and incidentally hemosiderin) may concentrate at any site of inflammation and that the synovial membrane is certainly not an exception in this respect. In fact, not uncommonly, one finds considerable amounts of lipoid in the synovial lining of a joint inflamed from rheumatoid arthritis, in regard to which again one cannot invoke cholesterol imbalance as the cause.

On the other hand, it was not found possible to reproduce experimentally anything resembling the complete picture of pigmented villonodular synovitis by an intra-articular injection of cholesterol. Cholesterol injected into a joint was rapidly absorbed into the synovial membrane, provoking nothing more than a mild foreign body reaction about the crystals. Cholesterol ester—e. g., cholesterol palmitate—was likewise rapidly absorbed from the joint and its presence was found to result merely in the appearance of small masses of foam cells in the synovial membrane. Cholesterol or cholesterol ester together with ferric chloride likewise failed to produce anything like the lesion.

Thus, altogether, the facts do not convince us that the lesion represents a true neoplasm. They point instead toward the view that it represents an inflammatory reaction. However, as pointed out, we do not hold that this inflammatory reaction is a response to anything quite as obvious as local hemorrhage or lipoid imbalance. Nor are we able to support by any positive evidence the idea that it has an infectious basis of one sort or another. Indeed, against this idea we can cite, for what it is worth, the fact that in all those of our cases in which cultures were made of material from these lesions they proved negative for bacteria. And still the whole cellular response dynamizing the development of the lesion—a response centering around the accumulation of macrophages and the assumption of phagocytic qualities by them—does suggest inflammation. This accounts for our use of the suffix "itis" in our designation of the lesion in its various sites, but we realize that by itself the idea of inflammation does not carry us very far toward understanding the causes of the condition.

TREATMENT AND PROGNOSIS

The appropriate treatment for the lesion in any of its sites is surgical excision. If the lesion can be removed in its entirety, no recurrence is to be expected. Complete removal is usually possible in connection with

the smaller lesions of tendon sheaths, the circumscribed lesions in synovial membranes and lesions of bursae. When such lesions of tendon sheaths and synovial membranes do recur, there is a tendency to attribute the recurrence to incomplete excision. However, one may be dealing with a new and independent lesion which developed in the same general area as the extirpated lesion. Indeed, it would seem to be sound practice when removing such a lesion from a tendon sheath or a lesion of the circumscribed type from a synovial membrane to excise it with a margin of apparently uninvolved sheath or synovial membrane. If this procedure is used, the incidence of recurrence, small though it is, will probably be reduced still further.

As one might expect, if the lesion winds over and between the tendons of a wrist or an ankle or is diffused over the entire synovial membrane of a knee joint, removal is not infrequently followed by recurrence, on account of the difficulty of complete extirpation. The diffuse pigmented lesion of the synovial membrane in particular has a distinct tendency to recur, even within a few weeks or months after synovectomy has been done. The recurrent lesion is likely to be somewhat more florid than the original one. However, it has become apparent that the recurrences yield well to roentgen therapy. Conceivably, the latter may be effective by causing obliteration of blood vessels and by augmenting the spontaneous tendency toward fibrosis and hyalinization. We have observed 2 cases of involvement of the knee joint in which recurrences were followed by remarkable amelioration, if not permanent cure, under roentgen therapy.[17] There is every reason to suppose that the latter would have a beneficial effect on the original lesion, also, if only it were diagnosed before operation.

17. M. Friedman and A. Ginzler (Bull. Hosp. Joint Dis. **1**:17, 1940) have given details of the therapy used in one of these cases, and the results, as follows: "X-ray therapy was given with the following factors: —200 K. V.; 20 M. A.; 50 cm. distance; 10× 15 cm. field; ½ mm. Copper and 1 mm. Aluminum filter. The treatment was administered in divided doses from Jan. 17, 1938 to Feb. 9, 1938. The total dose was: Anterior right knee —1600 'r' (measured with back scattering), posterior right knee —1600 'r' (measured with back scattering). On Feb. 7, 1938, the swelling had subsided considerably, especially that in the suprapatellar region. The diameter of the right knee was 2.2 cm. greater than the left. She could walk for several miles, and could flex her knee 60 degrees. Because of some residual swelling in the suprapatellar region, a second course of x-ray therapy was given from April 7, 1938 to May 12, 1938. The dose was: Anterior right knee —1600 'r' (measured with back scattering), posterior right knee —1600 'r' (measured with back scattering). In July 1938, she was able to resume all activities including dancing. There was mild synovial thickening; the patella was freely movable. There was normal extension and flexion to 80 degrees. In February 1939, there were no complaints. She was able to engage in all activities, such as dancing and walking several miles, and there was only very slight limitation of extreme flexion. The circumference of both knees was equal (14 inches)."

SUMMARY

Under the heading "villonodular synovitis, bursitis and tenosynovitis" we have developed our conception of the linkage between (1) the tenosynovial lesion which has commonly been denoted as xanthoma, xanthogranuloma, giant cell tumor or myeloplaxoma of the tendon sheath and (2) the synovial and bursal lesions, likewise often classified under such heads but also in certain forms (at least so far as the synovial membrane is concerned) under other headings, viz., "chronic hemorrhagic villous synovitis," "giant cell fibrohemangioma," "fibrohemosideric sarcoma," "sarcoma fusogigantocellulare," "benign polymorphocellular tumor of the synovial membrane" or "malignant polymorphocellular tumor of the synovial membrane."

In 9 of 20 cases the involvement was circumscribed and the affected portion of the synovial membrane showed one or more yellow-brown sessile or stalked tumor-like nodular outgrowths of the type commonly referred to as xanthoma, giant cell tumor and so on. In 11 cases the involvement was diffuse and the affected synovial membrane was brownish and appeared bearded with delicate, though somewhat matted, villi, showed villi intermingled with numerous coarse nodular outgrowths or presented an even more complex pattern through intergrowth of villi or of villi and nodules. It is to such diffuse synovial lesions that the names just noted, other than "xanthoma" or "giant cell tumor," have often been applied. Our experience with the bursal lesion comprises 4 instances of diffuse pigmented villonodular involvement. Among the 55 instances of so-called xanthoma, giant cell tumor, etc., of tendon sheaths were several which shed light on the evolution of these lesions and which in this respect clearly linked them with villonodular synovitis and bursitis.

As to the causation and nature of the condition, we reject, largely on the basis of the cytologic findings, the interpretation of the lesion as a true tumor and favor the theory that the condition represents an inflammatory response, though we do not know to what agent.

From the clinical point of view, emphasis is laid on the predilection of pigmented villonodular synovitis for the knee joint and of pigmented villonodular tenosynovitis for the region between the wrist and the finger tips. As a diagnostic criterion of diffuse villonodular synovitis of the knee joint, emphasis is laid on the presence of serosanguineous fluid on aspiration, and especially on repeated aspiration.

As to treatment of the lesion in any site, surgical excision is stressed as appropriate. Notice is also taken of the value of roentgen therapy in the prevention of or treatment of recurrences, particularly those of diffuse lesions of synovial membranes but also recurrences at other sites when the lesions cannot be, or have not been, completely removed.

HISTOLOGIC DISTRIBUTION OF VITAMIN A IN HUMAN ORGANS UNDER NORMAL AND UNDER PATHOLOGIC CONDITIONS

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With the aid of fluorescence microscopy, vitamin A can be visualized in tissues because of its specific fluorescence. The purpose of this communication is to describe the distribution of the vitamin A fluorescence in human tissues. The knowledge of the distribution of this fluorescence may be applied (*a*) to the study of vitamin A metabolism and (*b*) to the use of this fluorescence as a specific means of histologic examination, independent of its physiologic significance. The fluorescence microscopic pictures of various organs in fresh frozen sections will be discussed, especially as to the distribution of the lipoids and pigments. This description augments and partially corrects reports of Hamperl [1] and others based on embedded or less freshly studied material.

Paraplasmatic inclusions that imparted a fluorescence fading on irradiation were observed by Querner [2] in epithelial cells of the liver, the adrenal cortex and the pituitary gland. Since he found a similar fluorescence in vitamin A concentrates, he assumed, on the basis of animal experiments, that this fluorescence in organs was due to the presence of vitamin A. Hirt and Wimmer [3] reported a similar fluorescence observed in microscopic examinations of tissues of living animals. Jancsó [4] found this fluorescence in the retina. It has been shown [5] that a characteristic green fluorescence of the livers and adrenals of normal animals, which is removed by lipoid solvents, is absent in those of vitamin A–deficient animals. After administration of vitamin A to deficient animals the fluorescence reappears and the amount of the

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fluorescence is in direct proportion to the given dose of vitamin A and to the quantities of vitamin A found chemically in the liver. In nutritional deficiencies other than vitamin A deficiency a normal amount of the green fluorescence is observed. Further investigations [6] on avitaminotic and hypervitaminotic rats, with various feeding experiments and chemical analyses, gave added evidence that this green fading fluorescence in fixed tissues is specific for vitamin A. The distribution of vitamin A in the human liver was briefly described by Schairer and co-workers [7] and simultaneously by me.[8] The results from the fluorescence microscopic analyses are not in agreement with those from the histologic application of the antimony trichloride reaction for vitamin A.[9]

METHODS AND MATERIAL

Since the method of the visualization of vitamin A in tissues by means of fluorescence microscopy has not been described in every detail heretofore, the following description is presented.

Microscope.—Fluorescence microscopy is discussed in some recent monographs.[10] I used the fluorescence microscopic apparatus of Reichert, Vienna, Austria, and the fluorescence lamp of Pfaltz and Bauer, New York.

A mercury vapor bulb was used as a source of light. The light, rich in ultraviolet rays, was filtered through an ultraviolet filter, corresponding to the Corning glass color filter no. 584, in order to eliminate the visible light. To absorb the residue of the red rays, a glass cell containing a 5 per cent cupric sulfate solution, or a Corning glass color filter no. 428, was used. It was possible to change the ultraviolet filter to a ground glass filter which permitted observation in visible light. To observe the same field under polarized light, it was only necessary to insert Nicol prisms on the microscope.

A standard microscope with a quartz mirror was used. For the production of the fluorescence in tissue sections and especially for the visualization of vitamin A, only the longer wavelengths of the ultraviolet, near the visible violet, are utilized. These long waves are not absorbed by glass. Therefore, the standard Abbé condenser was used.

Some ultraviolet rays which strike the tissue section are changed to visible light of different colors—fluorescence. The latter is observed. The remainder of the ultraviolet rays was cut out by a brown filter over the eye piece (Leitz ultraviolet protecting filter no. 8574A).

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9. Joyet-Lavergne, P.: Protoplasma **28**:131, 1937; Compt. rend. Acad. d. sc. **200**:346, 1935.

10. (*a*) Radley, J. A., and Grant, J.: Fluorescence Analysis in Ultraviolet Light, ed. 3, New York, D. Van Nostrand Company, 1939. (*b*) Haitinger, M.: Fluorescenz-Mikroskopie, Leipzig, Akademische Verlagsgesellschaft, 1938.

The fluorescence microscope yields a dark field, and only fluorescing material imparts the visible picture. Nearly all tissue constituents reveal some kind of fluorescence; e. g., collagenous fibers show a strong blue, elastic fibers a gray, fluorescence. The orientation in an unstained section under the fluorescence microscope is as readily accomplished as it is in a stained section with visible light.

Photomicrographs were taken with a 35 mm. camera.[11]

Fixing and Sectioning.—Solution of formaldehyde U. S. P. (1: 10) proved to be the most applicable fixative; fixing mediums containing fat solvents, metals and trinitrophenol remove vitamin A or interfere with fluorescence. Striking changes, especially of the lipoids, were noted under ultraviolet rays during prolonged formaldehyde fixation.[12] The vitamin A fluorescence was affected by formaldehyde within ten to forty-eight hours. The rapidity of change and the final disappearance varied with different organs and conditions. The fluorescence was altered sooner in the adrenal than in the liver and disappeared very early in a case of myelogenous leukemia. Refrigerator temperature preserved the fluorescence better than room temperature. Incubation at 37 C. caused the fluorescence to disappear faster.

The tissue blocks were not more than 3 mm. in thickness and were fixed in an excess of solution of formaldehyde. The frozen sections were not preserved longer than three hours before observation.

The rate of the change of the fluorescence was not the same in different sites in the same organ. For example, in the liver the fluorescence disappeared first from the Kupffer cells, then from the lipoid droplets and much later from the lipofuscin. At times it appeared in places where it was not visible originally. The fluorescence in the Kupffer cells eventually disappeared, and a green fading fluorescence was manifested in lipoid droplets which previously did not exhibit this fluorescence. This was due to a postmortem imbibition of vitamin A by the lipoid droplets and was reproduced if a section of a liver rich in vitamin A and one of a vitamin A–free adrenal were bathed together in 1 cc. of methyl alcohol. The epithelial cells of the adrenal acquired a slight green fading fluorescence. This did not occur if the adrenal alone was treated with methyl alcohol.

The vitamin A fluorescence was visible in fresh, nonfixed material; but the preparation of sections was difficult.

The common mounting mediums for frozen sections either yielded a disturbing fluorescence (glycerin) or dissolved the vitamin A (mineral oil). The sections, therefore, were observed in water. It was not possible to save the sections for future study.

Observation.—The rapidity of the fading of the vitamin A fluorescence varied directly with the amount of vitamin A present. Minute quantities faded completely within ten seconds after exposure to the ultraviolet rays. The fluorescence of the normal liver faded within forty-five seconds; in hypervitaminosis more than a minute was required.[6] The thickness of the section was also significant. After the fading of the green fluorescence, a dim blue fluorescence of the cytoplasm and a definite blue fluorescence of the fat droplets remained. Vitamin A may be carried by other fluorescent substances, such as the lipofuscin in the liver and the brown-red fluorescent pigments in the Leydig cells of the testicle or in the theca cells of the corpus atreticum of the ovary. The innate fluorescence of the substances just named was less striking than the fluorescence of vitamin A and was visible only

11. Popper, H., and Elsasser, M.: Am. J. M. Technol. **3**:45, 1941.

12. Haitinger, M., and Hamperl, H.: Ztschr. f. mikr.-anat. Forsch. **33**:194, 1933.

after the disappearance of the vitamin A fluorescence under irradiation or under treatment of the sections with alcohol. Other green fluorescent substances were differentiated from vitamin A by their stability in ultraviolet rays.

Without the Abbé condenser, the fluorescence was weaker, but the fading was slower and started only after irradiation for eight minutes. The fading in visible light was still slower.

Irradiation with ultraviolet rays not only destroyed the vitamin A fluorescence but also modified the color reactions with antimony trichloride and the biologic activity.[13]

Chromolysis.—The chromolytic experiments showed a similar behavior of the fluorescence in the droplets of vitamin A concentrates[14] and of the fluorescence in the tissue constituents. They further demonstrated identical reactions of the green fading fluorescence in rats and human organs. The experiments on rats[15] conclusively showed that the green fading fluorescence in rat organs was due to the presence of vitamin A. The chromolytic experiments supported the same finding in human organs.

Tissue sections of livers and adrenals from human subjects and normal rats were examined and compared with six different vitamin A concentrates and with different samples of cod liver oil. The oils and concentrates were examined in thin aqueous emulsions. They were also injected intratracheally into mice; the lungs were fixed and frozen sections were examined to demonstrate the fluorescent material in the alveolar lumens.

The green fading fluorescence was not changed by tenth-normal hydrochloric acid, tenth-normal ammonium hydroxide or saturated sodium hydrosulfite solution (reducing agent).[16] The stability of the fluorescence in reducing agents differentiated the vitamin A fluorescence from the similar fluorescence of vitamin B_2. The latter was quickly destroyed by reducing agents. Hydrogen peroxide did not change the fluorescence of the concentrates or of the tissues. The green fluorescence was somewhat concealed by the striking blue fluorescence of the oxidized fat.

In the tissues ethyl alcohol, methyl alcohol, acetone, xylol and liquid petrolatum dissolved the fluorescent substance. The latter was also dissolved after prolonged treatment with linseed oil, peanut oil, rancid lard or oleic acid, but was insoluble in glycerin.

The speed of the dissolution of the fluorescent substance in alcohol varied. It depended on the dissolution of the substances acting as carriers of vitamin A.

Fluorochromy.—The study of the spontaneous fluorescence was extended by examination of sections stained with fluorescent dyes (fluorochromy, Haitinger[10b]).

Staining for ten seconds in 1 per cent aqueous methylene blue produced a bluish fluorescence of medium-sized or larger lipoid droplets in a nonfluorescent back-

13. Peacock, P. R.: Lancet **2**:328, 1926.

14. The Abbott Laboratories and Mead Johnson & Co. furnished the vitamin A concentrates. Recently I studied under the fluorescence microscope crystalline vitamin A alcohol and crystalline vitamin A acetate furnished by Distillation Products, Inc., Rochester, N. Y. (see Baxter, J. G., and Robeson, C. D.: Science **92**:203, 1940). Both were dissolved in corn oil. The droplets of an oil in water emulsion made from these solutions revealed the same green fading fluorescence as emulsions made from vitamin A concentrates. The fluorescence was present up to dilutions which were equivalent in international units to the highest dilutions of the concentrates giving fluorescence.

15. Popper.[5] Popper and Greenberg.[6]

16. von Querner, F., and Sturm, K.: Anat. Anz. **78**:289, 1934.

ground. The green vitamin A fluorescence surpassed the blue fluorescence and was distinctly visible. After fading of the vitamin A fluorescence, the vitamin A–containing fat yielded the same bluish fluorescence as the vitamin-free fat. Thus fats that carried vitamin A were differentiated from fats which were free of vitamin A. Methylene blue concealed most of the other types of green fluorescence, which were not due to vitamin A; this facilitated a differentiation between the green types of fluorescence. If the ultraviolet filter was changed to a ground glass filter, the section which was stained with methylene blue could be observed in visible light. Thus the fluorescence was localized in visible light.

Haitinger mentioned several fluorescent fat stains. My investigations indicate that the produced fluorescence varies with the time of fixation. After formaldehyde fixation of a few days, an irregular fluorescence of the fats was obtained on staining. Such changes were extensive in liver and adrenal.

If the fixation time was not over twelve hours and water was used as a mounting medium, a reliable lipoid-staining effect was obtained by use of a 0.1 per cent aqueous phosphin 3R solution for three minutes. A very distinct silver white fluorescence appeared in a brown background. With phosphin 3R in ultraviolet rays, more lipoids were visualized than with the methods employing visible light, and the picture was clearer. The specificity of the reaction for lipoids was proved by the absence of the silver white fluorescence in sections previously treated with acetone. The phosphin 3R fluorescence did not depend on the presence of vitamin A, although sometimes vitamin A and phosphin 3R fluorescence ran parallel; the latter phenomenon indicated that all lipoids present carried vitamin A. But more often, not all lipoids visualized with phosphin 3R imparted vitamin A fluorescence. The phosphin 3R fluorescence was produced in areas where the vitamin A fluorescence had been destroyed previously with ultraviolet radiation. The strong phosphin 3R fluorescence masked the vitamin A fluorescence.

A 0.1 per cent solution of thioflavine S produced a violet fluorescence of fat droplets in a yellow background within ten minutes. The vitamin A fluorescence surpassed the violet fluorescence. Less lipoids stained with thioflavine S than with phosphin 3R.

By simple staining procedures, none of the other dyes mentioned by Haitinger, such as chlorophyll, neutral red, chrysarobin or extract of rhubarb, gave a reliable result in material fixed in solution of formaldehyde for a short time. Magdala red or bengal rose (0.1 per cent solution for one minute) produced a good picture of the background without disturbing the vitamin fluorescence. This also facilitated the localization of vitamin A.

Observation.—The fluorochromic examinations revealed that vitamin A was carried by lipoids which with few exceptions were demonstrable by phosphin 3R. Not all these lipoids, however, were visualized by methylene blue or thioflavine S. The tissue constituents which with the fluorescence microscope were observed to show green fading fluorescence were not vitamin A per se but lipoids which carried vitamin A and were thus made fluorescent. Consequently, vitamin A serves as a vital stain for some lipoids.

Material.—The material studied was obtained from autopsy and biopsy specimens.

LIVER

The liver was examined in 240 autopsy cases. The green fading fluorescence was found in the Kupffer and epithelial cells.

Distribution in the Kupffer Cells.—In the Kupffer cells vitamin A fluorescence was noted as droplets which filled the cytoplasm, thus imparting an outline of the characteristic stellate form of the Kupffer cell (fig. 1 *A, B* and *D*). Under normal conditions the Kupffer cells frequently revealed lipoid droplets, which were demonstrable with sudan III (Levine [17]) and especially clear with phosphin 3R. These droplets imparted the vitamin A fluorescence. In several cases, after fading of the green fluorescence a dim blue spontaneous fluorescence of the lipoids remained, indicating a high fat content.

In a few cases inclusions showing brown fluorescence, stable under ultraviolet irradiation, appeared, which apparently were lipofuscin. These inclusions were seen in the vicinity of liver cells which were rich in lipofuscin. Lipofuscin in Kupffer cells was found also in enlarged periportal fields, in pseudolobules and in cirrhotic livers. Occasionally, pigments in Kupffer cells interfered with the presence of vitamin A. In a case of malaria no vitamin A fluorescence was seen. If bile pigment was stored in the Kupffer cells, as in severe jaundice, the vitamin A fluorescence was reduced. In cells with iron storage no vitamin A fluorescence was visible. Other brown pigments usually did not interfere with the vitamin A fluorescence. Malaria pigment, iron pigment and concentrated bile pigment per se were not fluorescent. Proliferated Kupffer cells were commonly rich in vitamin A fluorescence.

In the normal liver the distribution of the vitamin A fluorescence was uniform throughout the lobules. With a heterogeneous distribution of the Kupffer cell fluorescence, striking variation in the vitamin fluorescence of the liver cells was present or some pathologic change of the liver existed. There was corresponding vitamin A fluorescence in Kupffer cells and adjacent liver cells. In areas where the liver cells showed increased vitamin A fluorescence, the neighboring Kupffer cells were also rich in the fluorescence. Where the liver cells were free of vitamin A, the Kupffer cells were also free, even when the latter were proliferated.

Divergence indicated pathologic change. In toxic hepatic edema, diabetes mellitus, jaundice or damage to the liver parenchyma of minor degree, the Kupffer cells were rich in vitamin A fluorescence, while the liver cells were free. Presence of vitamin A in the liver cells and absence from the Kupffer cells constituted a rare finding which was encountered only in cases in which the vitamin A content of the liver was very much reduced, as in malnutrition or in severe damage of the parenchyma of the liver. In these cases vitamin A fluorescence was present only in the larger fat droplets of the liver cells.

17. Levine, V.: Arch. Path. **14**:345, 1932.

Distribution in Epithelial Cells.—In the epithelial cells vitamin A fluorescence was found at four sites. It was seen in the larger fat droplets, which were clearly demonstrated by the common stains for fat (fig. 1 *C*). The fluorescence was not brilliant. The stronger the fluorescence the smaller were the fat droplets. The degree of the fluorescence occasionally varied in droplets of the same cell.

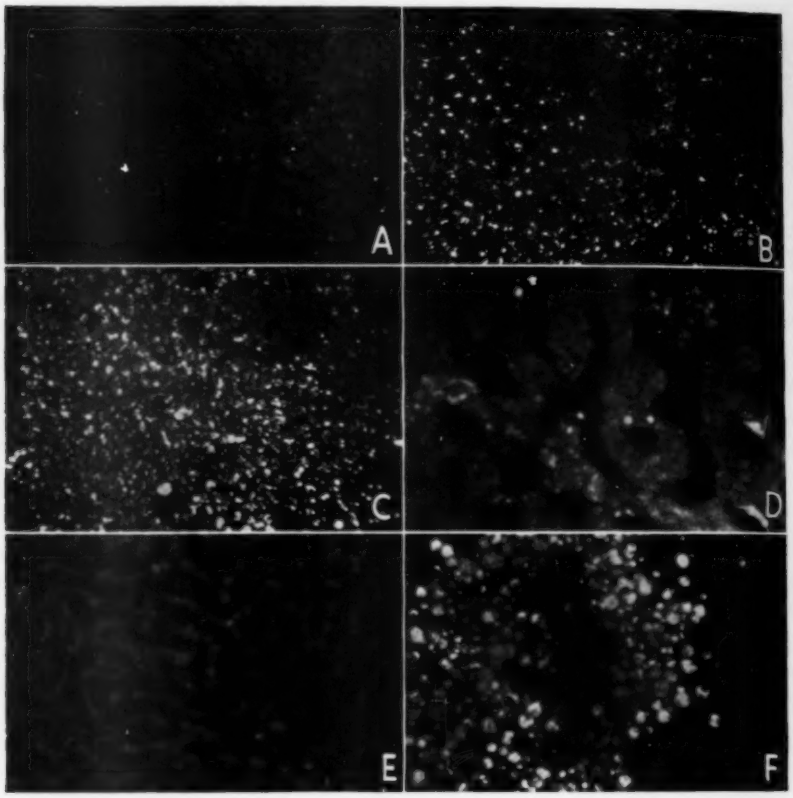

Fig. 1.—Fluorescence photomicrographs: *A*, liver poor in vitamin A fluorescence, which is imparted by Kupffer cells and by lipofuscin (esophageal carcinoma). *B*, normal liver showing vitamin A fluorescence in Kupffer cells and in fine droplets in the liver cells, especially on the edges of cells. *C*, normal liver rich in vitamin A fluorescence, which is imparted by small and medium-sized droplets in the liver cells and by the Kupffer cells. *D*, normal liver under high power magnification, showing vitamin A fluorescence of the Kupffer cells and of fine lipoid droplets lining the edges of the liver cells. *E*, vitamin A fluorescence imparted by lipofuscin in the center of the liver cell cords. *F*, local fatty infiltration in the liver. The large fat droplets are rich in vitamin A fluorescence.

Vitamin A was present in small lipoid droplets which were not distinctly visualized by sudan III but were made strikingly fluorescent

by phosphin 3R (fig. 1 *B* and *D*). These droplets were irregularly distributed in the cytoplasm and often filled it almost completely; or they alined themselves regularly, with uniform vitamin A fluorescence, at the edges of the cells, like pearls on a string. The latter distribution gave a picture closely resembling that in the livers of normal animals.[6] Cells with large fat droplets did not contain the finer droplets.

Irregular lumps in the centers of the cells often imparted vitamin A fluorescence (fig. 1 *A* and *E*). These lumps appeared in adults in amounts increasing with age, especially around the central vein. After the fading of the vitamin fluorescence or after treatment with alcohol, these lumps revealed a weaker red fluorescence. According to their localization and form, they represented "wear and tear" pigment or lipofuscin.[1] Lipofuscin did not always carry vitamin A, even if the latter was seen in other parts of the liver cells; it gave the phosphin 3R reaction only when high amounts of vitamin A were present.

A diffuse green weak fading fluorescence, not present after treatment with alcohol, was imparted by the cytoplasm. This indicated the presence of relatively small amounts of vitamin A, which was diffusely spread over the cytoplasm and was not bound to microscopically demonstrable lipoids. There were other forms of green but ultraviolet-stable fluorescence in the cytoplasm. Bile pigment gave a diffuse green or brown fluorescence. In icterus neonatorum the bile pigment produced a red fluorescence, indicating a different nature of the bilirubin. Concentrated bile pigment, however, as in Kupffer cells or in casts of the bile capillaries, showed no fluorescence.

In distribution and amount the vitamin A fluorescence of the liver cells, even in morphologically normal organs, was quite variable. It varied within the lobules. The central as well as the peripheral zone was often free of vitamin A. In the latter the cells revealed a dark homogeneous cytoplasm in visible light. In areas of local fatty infiltration the large fat droplets imparted a strong vitamin A fluorescence (fig. 1 *F*).

Estimation of the Total Amount of Vitamin A Fluorescence.—Several major tendencies were apparent in the variations of the amount of the vitamin A fluorescence. Table 1 gives estimations of the total amounts of the vitamin A fluorescence of the examined livers of adults, grouped according to diseases. One can safely assume that the amounts in accidental death, cerebral disease, organic heart disease, abdominal disease and tumor without emaciation approached the normal. A pathologic reduction was found in malnutrition resulting from tumor, such as carcinoma of the gastrointestinal tract. The liver of a pregnant woman who died from pernicious vomiting showed only traces of vitamin A fluorescence. In the other cases of pregnancy the fluorescence was normal.

More striking was the reduction of vitamin A fluorescence in disease of the liver per se, such as acute hepatitis and cirrhosis. While there was a slight reduction in livers from persons with compensated cirrhosis, livers from those with decompensated cirrhosis (with superimposed

TABLE 1.—*Estimation of the Amount of Vitamin A Fluorescence in Livers of Adults, Grouped According to Diseases*

Diagnosis	Livers Showing Estimated Amount of Fluorescence					Total
	0	±	+	++	+++	
Accidental death	..	..	..	2	..	2
Cerebral disease	..	..	..	4	3	7
Abdominal disease	..	..	2	5	5	12
Heart disease	..	..	4	9	2	15
Pulmonary disease	..	..	2	6	..	8
Tumor without emaciation	..	..	..	12	..	12
Tumor with emaciation	..	3	2	9	1	15
Renal disease	..	1	8	12	1	22
Pulmonary tuberculosis	..	1	4	2	..	7
Blood disease	..	1	5	5	..	11
Sepsis and infectious disease	..	..	6	5	..	11
Diabetes mellitus	..	1	1	4	1	7
Pregnancy	..	1	1	1	1	4
Obstructive jaundice	..	4	3	2	..	9
Acute hepatitis	..	2	3	..	..	5
Compensated cirrhosis of the liver	..	1	4	5	..	10
Decompensated cirrhosis of the liver	..	12	..	..	..	12
Hepatocellular carcinoma with cirrhosis	..	2	..	1	..	3
Thrombosis of the portal vein	..	..	..	1	..	1
Amyloidosis	..	1	2	..	..	3

TABLE 2.—*Estimation of the Amount of Vitamin A Fluorescence in Livers of Fetuses and Children Below Ten Years of Age*

Age Group	Livers Showing Estimated Amount of Fluorescence					Total
	0	±	+	++	+++	
Fetal	..	..	..	4	..	4
Premature	1	8	2	1	..	12
Stillborn	..	3	..	..	..	3
Newborn (first day)	1	2	2	..	..	5
1 month	1	2	..	1	..	4
2 months	..	1	2	1	..	4
3-4 months	..	2	3	3	..	8
5-6 months	1	..	1	1	..	3
7-12 months	..	2	3	6	..	11
2-8 years	..	1	..	9	..	10

acute hepatitis), with or without jaundice, revealed only traces of vitamin A fluorescence. In the examined livers of persons with obstructive jaundice a secondary damage of the hepatic tissues was present.

Another important factor was age. As seen in table 2, in the fetus at from 4 to 6 months considerable amounts of vitamin A fluorescence appeared in Kupffer and liver cells. The mother of one fetus died shortly after abortion from acute atrophy of the liver, her liver being nearly depleted of vitamin A.

Toward term a reduction of the vitamin A storage occurs, only traces being encountered in premature, stillborn and newborn infants, without influence of the degree of maturity. The small amounts were equally distributed over Kupffer and liver cells. The foci of hemopoiesis were free. Infants who died during the first month of postnatal life showed small amounts. A rise to the normal started in the second month, and after the second year the storage resembled that of adults. The influence of disease on the fluorescence was not as evident in children's livers as in those of adults, but similar trends were apparent. Complete absence was found in 4 livers: 1 from a premature infant, 2 from infants with syphilitic interstitial hepatitis and 1 from an infant with congenital atresia of the bile passages. In the latter 3 livers the parenchymatous damage was significant.

Neither premortal fever nor the terminal state of nutrition nor the interval between the last intake of food and death was significant.

Distribution in Pathologic Livers.—Extensive changes appeared in the distribution of vitamin A fluorescence in livers involved in pathologic conditions.

(*a*) Local Damage: A reduction or absence of vitamin A fluorescence was noted in areas showing various types of local damage of liver cells in contrast to the neighboring areas of intact tissue. A Zahn infarct (due to thrombosis of branches of the portal vein), hardly recognized under ordinary microscopic examinations, was free of vitamin A, whereas the neighborhood revealed an accumulation of the fluorescence in the Kupffer and liver cells (fig. 2*A*).

In central necroses the liver cells were free of vitamin A, although they contained lipoid droplets or lipofuscin or even showed fatty degeneration. The Kupffer cells often retained vitamin A; in more advanced stages they were free. The Kupffer cells of the adjacent tissue were extremely rich in the vitamin A fluorescence.

In the vicinity of extrinsically growing tumors the compressed liver cells were free of vitamin A fluorescence or revealed it in lipofuscin. A local accumulation of the fluorescence was visible if, owing to the atrophy of the cells, the lipofuscin filled almost the whole cytoplasm.

(*b*) Toxic Edema: In 24 cases the Kupffer cells imparted strong vitamin A fluorescence, but the liver cells were free of fluorescence, although they may have contained lipoids (fig. 2*B*). In 2 other cases some vitamin A fluorescence was seen in lipofuscin. In practically all these cases there was hepatic edema.[18] The interstitial spaces between

18. Rössle, R.: Entzündungen der Leber, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1930, vol. 5, pt. 1, p. 250. Popper, H.: Wien. klin. Wchnschr. **49**:207, 1936; Virchows Arch. f. path. Anat. **298**:574, 1937.

the liver cell cords and the sinusoids were widened and filled with albuminous material. The Kupffer cells were quite apart from the liver cells and often proliferating. The foregoing picture was encountered in infectious diseases, endocarditis, septic forms of pneumonia and in renal diseases, especially those with uremia. In tuberculosis various pictures were seen.

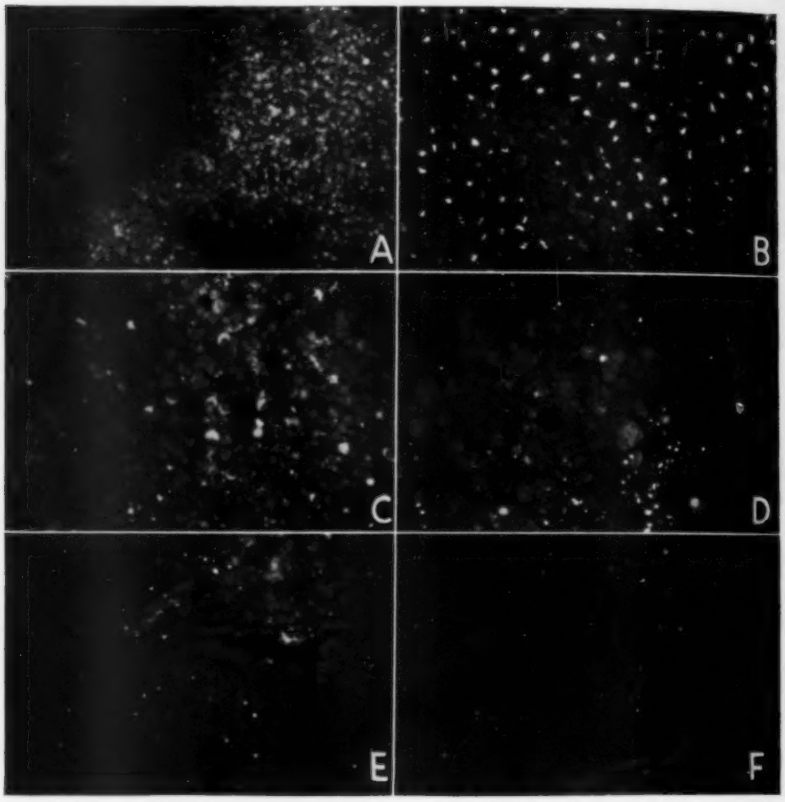

Fig. 2.—Fluorescence photomicrographs: *A*, Zahn's infarct of the liver (thrombosis of branches of the portal vein). The infarct is free of vitamin A fluorescence, whereas the surrounding intact tissue reveals an accumulation of vitamin A fluorescence. *B*, toxic edema of the liver. The Kupffer cells are rich in vitamin A fluorescence; the liver cells show traces. *C*, fatty liver in obesity. The fat droplets in the liver cells are rich in vitamin A fluorescence. *D*, fatty liver in acute hepatitis. There is little vitamin A fluorescence of the fat droplets in the liver cells. There is a bizarre distribution of the vitamin A fluorescence in the Kupffer cells. *E*, compensated cirrhosis of the liver. Moderate amounts of vitamin A fluorescence may be seen in the Kupffer cells. *F*, decompensated cirrhosis of the liver with superimposed acute hepatitis. There are traces of vitamin A fluorescence in the fat droplets.

In 7 cases a similar local edema was seen in some parts of the lobules. In the involved areas the liver cells failed to reveal vitamin A fluorescence, while the Kupffer cells were rich in it. In a case of pylephlebitic abscess, the Kupffer cells in the necrobiotic area were strongly fluorescent, whereas the liver cells were not; in normal areas both cells imparted the fluorescence.

In 3 cases of more advanced damage of liver cells, characterized by dissociation of the liver cell cords,[19] a similar picture was noted.

In diabetes mellitus the liver cells revealed some vitamin A fluorescence, especially in lipofuscin. The fluorescence of the Kupffer cells was very rich, out of proportion to that of the liver cells.

(*c*) General Hepatic Damage: In fatty livers without damage, as in obesity or tuberculous peritonitis, the Kupffer cells revealed normal fluorescence and especially the small fat droplets in the liver cells were rather rich in it (fig. 2*C*). In fatty livers with acute hepatitis (as seen in 2 cases of alcoholism) vitamin A fluorescence was strikingly reduced; the Kupffer cells showed none in 1 case and little, in irregular distribution, in the other (fig. 2*D*). The fat droplets in the liver cells revealed a green nonfading fluorescence, due to bilirubin in the fat. In contrast to local or general fatty infiltration, the larger fat droplets imparted even more vitamin A fluorescence than the smaller ones. Phosphin 3R demonstrated more fat than was expected from the vitamin A fluorescence in these cases.

In 3 other cases of acute hepatitis (without fatty livers) small amounts of vitamin A fluorescence were found. A diverse picture was seen with irregular fatty degeneration, the lipoids being free of vitamin A. Few of the vitamin A–free lipoid droplets showed a strong brown fluorescence, whereas most revealed a green bilirubin fluorescence. In places, large irregularly shaped lipoid droplets in Kupffer cells revealed vitamin A fluorescence.

In another case, that of a 7 year old boy who died shortly after the onset of the disease, central necroses and peripheral dissociation was seen. The central necroses and the Kupffer cells throughout failed to reveal the fluorescence, whereas the dissociated liver cells in the periphery showed it.

In a case of subacute atrophy of the liver the regenerating pseudolobuli were rich in bilirubin fluorescence but free of vitamin A. In the surrounding granulation tissue, vitamin A fluorescence was seen in the irregularly scattered liver cells and in the Kupffer cells, which appeared increased in number, owing to collapse of the liver tissue.

In 4 of 9 cases of obstructive jaundice the Kupffer cells showed considerable vitamin A fluorescence, while the liver cells imparted traces or none, although they were occasionally rich in lipoids. Signs of damage of the liver cells were present. The concentrated bile pigment in Kupffer cells and bile capillaries did not fluoresce, in contrast to the more dilute bile pigment in the liver cells.

19. Kirshbaum, J. D., and Popper, H.: Arch. Int. Med. **65**:456, 1940.

(*d*) Cirrhosis: Various abnormal fluorescent details were seen. In the periportal fields there were large irregular-shaped lumps of striking nonfading brown, yellow or red fluorescence. This fluorescence was not removed by lipoid solvents. A dark red fluorescence of the liver cells was visible at times, probably due to hematoporphyrin. A green or brown stable fluorescence of the liver cells due to bile pigment was seen even in absence of jaundice.

The vitamin A fluorescence varied with the activity of the cirrhotic process. Compensated cirrhosis showed in some areas a normal amount of vitamin A fluorescence (fig. 2*E*); in the pseudolobules little was found in fat or lipofuscin. In decompensated cirrhosis with superimposed acute hepatitis only a small part of the lipoids, stained with phosphin 3R, revealed vitamin A fluorescence (fig. 2*F*). Rarely the Kupffer cells imparted an irregular vitamin A fluorescence. On one occasion, free fat droplets with vitamin A fluorescence were seen in the area of central necrosis, as after rupture of the liver cells.

There was no vitamin A fluorescence in the enlarged periportal fields in decompensated cirrhosis. In compensated cirrhosis fine lipoid droplets in the lining of capillaries and in scattered and compressed liver cells imparted the fluorescence. Proliferated small bile ducts did not luoresce.

In a case of hemochromatosis there was a picture of compensated cirrhosis, with much brown fluorescent pigment in the liver cells. In a case of cirrhosis carcinomatosa the variation was prominent; the tumor tissue was free of the fluorescence, while the cirrhotic tissue presented the fluorescence of compensated cirrhosis. This was also seen in a case of thrombosis of the portal vein.

(*e*) Congestion of the Liver: Congested livers also yielded diverse pictures. In typical cases there were apparent three layers, which agreed with the macroscopic zones. In the center there was a zone of cyanotic atrophy, where the compressed liver cells contained a brown fluorescent pigment and at times lipoid droplets. No vitamin A fluorescence was present. In the middle zone the Kupffer cells yielded considerable vitamin A fluorescence, especially the proliferating ones around the inner zone. The liver cells were rich in small lipoid droplets, which presented strong vitamin A fluorescence. The peripheral zone was composed of large liver cells, which stained dark in visible light and were free of lipoids and vitamin A fluorescence; the Kupffer cells were free also.

There were many variations from this picture; but even in congestion of minor degree the vitamin A–free peripheral zone with the dark cells was visible. In 2 cases of cardiac cirrhosis a combination of the picture of congestion of the liver with that of compensated cirrhosis was seen. The liver cells showed green or brown fluorescence due to bile pigment.

(*f*) Infiltrations: Infiltrations of lymphocytes, of lymphatic or myelogenous leukemic character, and of Hodgkin's lymphogranulomatosis did not reveal any vitamin A fluorescence. In the latter, irregular ultraviolet-stable yellow inclusions were seen. On the edge of the infiltrations, proliferating Kupffer cells showed considerable fluorescence. A similar proliferation was seen around pylephlebitic abscesses and tumor metastases. The latter often showed irregular-shaped bodies of various types of stable fluorescence, but were usually free of vitamin A, except for some fluorescent endothelial cells which were scattered throughout and apparently were Kupffer cells.

In 3 cases of amyloidosis the amyloid itself revealed a dim blue, but no vitamin A fluorescence.

Comment.—Great variation in the amount and distribution of vitamin A fluorescence in human livers was found. This coincided with the chemical analyses presented by several authors.[20] Histologically, however, the quantitative variation was augmented by the variation in distribution.

Three factors influence the amount of vitamin A fluorescence, in agreement with various chemical analyses: malnutrition, age and damage of liver cells. There was a definite reduction in malnutrition, but the reduction was not as extensive as expected. Total absence of vitamin A fluorescence from the liver was never found in adults, even though malnutrition had existed for some time, as in carcinoma of the gastrointestinal tract. A reduced intake of food previous to death was insignificant. This was not surprising, since it has been shown in adult rats that depletion of vitamin A depots requires many months.[6]

The slight vitamin A fluorescence in the livers of newborn and older infants coincided with the various chemical analyses [21] and with experiences in the study of animals.[6] No clearcut explanation can be given for the reduction of the considerable vitamin A depots of the fetus at the time of birth. Pregnancy did not significantly influence the vitamin A depots in the mother.[22] We saw low amounts in the newborn infant's liver when the maternal liver had normal amounts.

The reduction of the vitamin A depots in the presence of damage to liver cells, especially in cirrhosis, was demonstrated by chemical assays [23] and by clinical signs of hypovitaminosis, like hemeralopia.[24] If pathologic change of the liver occurred in an infant, vitamin A was com-

20. (*a*) Moore, T.: Biochem. J. **31**:155, 1937. (*b*) Schairer and others.[7]

21. (*a*) Ellison, J. B., and Moore, T.: Biochem. J. **31**:165, 1937. (*b*) Debré, R., and Busson, A.: Compt. rend. Soc. de biol. **114**:1164, 1933. (*c*) Wolff, L. K.: Lancet **2**:617, 1932.

22. Green, H. N.: Lancet **2**:723, 1932. Williams, P. F.; Hark, B., and Fralin, F. G.: Am. J. Obst. & Gynec. **40**:1, 1940. Hirst, J. C., and Shoemaker, R. E.: ibid. **40**:12, 1940.

23. Breusch, F., and Scalabrino, R.: Ztschr. f. d. ges. exper. Med. **94**:569, 1934. Cox, A. J.: Am. J. Path. **15**:647, 1939. Moore.[20a]

24. Patek, A. J., Jr., and Haig, C.: J. Clin. Investigation **18**:609, 1939.

pletely absent. Blackfan and Wolbach [25] observed morphologic signs of vitamin A deficiency in congenital atresia of the bile passages. That damaged liver cells usually did not reveal any vitamin A fluorescence was evidenced in local damage, where a comparison was possible with intact liver tissue. In such cases the lipoids may have been absent also. In instances of damage of liver cells, lipofuscin and large fat droplets apparently retained the vitamin A fluorescence longer.

In some cases of definite acute damage of liver cells, however, the liver cells imparted vitamin A fluorescence and vitamin A was also found chemically. This may be explained on the basis that in these examples the elicitation of the vitamin A from the liver cells was inhibited either by dissociation of the liver cell cords or by insufficiency of the Kupffer cells.

The histologic picture points to an interrelationship between Kupffer and liver cells in vitamin A metabolism. In irregular distribution of vitamin A fluorescence, the phenomenon in the liver cells was concomitant with that in the Kupffer cells. Sometimes the relation was enhanced by an aggregation of the vitamin A fluorescence in that part of the liver cells which was adjacent to the Kupffer cells.

The Kupffer cells are known to play an important role in lipoid metabolism. According to Jaffé and Berman,[26] lipoids injected intravenously are taken up by the Kupffer cells and immediately transmitted to the liver cells. The significance of the Kupffer cells for vitamin A metabolism [27] was shown by the absence of vitamin A storage in animals after blockage of the reticuloendothelial system.[28] Experiments on rats [6] revealed that the Kupffer cells are the last to lose vitamin A as a result of depletion and the first to store it in repletion of deficient animals. They probably split carotene and also hold the excess of vitamin A in hypervitaminosis.

The study of Kupffer cells in general is facilitated by the fluorescence microscopic examination for vitamin A and lipoids. A close connection between the Kupffer cell lipoids and vitamin A is suggested, because the lipoids of the liver cells may be free of vitamin A whereas those in the Kupffer cells invariably show some fluorescence. If some areas of the liver, as in central necroses or infiltrations, lose their vitamin A, the

25. Blackfan, K. D., and Wolbach, S. B.: J. Pediat. **3**:679, 1933. Altschule, M. D.: Arch. Path. **20**:845, 1935.

26. Jaffé, R. H., and Berman, S. L.: Arch. Path. **5**:1020, 1928.

27. Uotila, U. S., and Simolas, P. E.: Virchows Arch. f. path. Anat. **301**:523, 1938. Hirt and Wimmer.[3]

28. Lasch, F., and Roller, D.: Klin. Wchnschr. **15**:1636, 1936. Wendt, H., and König, D.: ibid. **16**:1253, 1937. Thiele, W., and Nemeth, K.: ibid. **18**:95, 1939.

neighboring Kupffer cells, which are usually proliferated, show increased vitamin A fluorescence. A similar explanation is possible for the picture of increased vitamin A fluorescence in the Kupffer cells with absence from the liver cells, as seen in certain forms of hepatic damage—in the peripheral zone of pylephlebitic abscesses or in tissues involved in toxic edema. A loss of vitamin A from the liver cells to the Kupffer cells has to be assumed, but also an inability of transmission from the Kupffer cells to the liver cells, in consideration of the animal experiments mentioned. As proposed,[29] a block between the Kupffer and the liver cells is indicated by the histologic picture. Besides the known reticuloendothelial block between the blood stream and the Kupffer cells, a Kupffer cell–liver cell block presents far reaching significance.

Whether this block in toxic edema of the liver, with absence of vitamin A from the liver cells, causes vitamin A deficiency is not certain, but it explains the low vitamin A stores found chemically in diseases sometimes associated with edema of the liver—septic or infectious diseases,[30] renal diseases [31] and tuberculosis.[32] In diabetes mellitus, supposedly associated with vitamin A deficiency,[33] the liver cells show moderate vitamin A fluorescence, whereas the Kupffer cells are extremely rich. This substantiates the chemical findings.[31] The high fat content of the Kupffer cells in diabetes mellitus is well known.[34]

In severe damage of the liver the Kupffer cells are free of vitamin A and lipoids or show an irregular, bizarre distribution which is characteristic for storage in damaged reticuloendothelial cells.

Besides the inability of damaged liver cells to store vitamin A, other causes have to be considered for the reduction of the vitamin A fluorescence of damaged livers, especially that observed in chronic hepatic diseases: (1) faulty absorption of vitamin A from the intestinal tract, as indicated by low blood levels in hepatic diseases [35] (bile acids are necessary for the absorption of carotene but not for that of vitamin A itself [36]); (2) inability (probably of the Kupffer cells) of the damaged liver to split carotene to vitamin A [37]; (3) a block in the transmission of vitamin A from the Kupffer cells to the liver cells; (4) increased demand for vitamin A in hepatic diseases.[38]

The study of the vitamin A fluorescence as histologically characterized permits an additional observation: Lipofuscin or "wear and tear" pigment of the liver is

29. Bauereisen, E.: Ztschr. f. d. ges. exper. Med. **103**:145, 1938.
30. Fox, F. W.: Lancet **1**:953, 1933. Moore.[20a]
31. Moore.[20a] Wolff.[21c] Breusch and Scalabrino.[23]
32. Crimm, P. D., and Short, D. M.: Ann. Int. Med. **13**:61, 1939.
33. Brazer, I. G., and Curtis, A. C.: Arch. Int. Med. **65**:90, 1940.
34. Rössle, R.: Verhandl. d. deutsch. path. Gesellsch. **11**:334, 1907.
35. Breese, B. B., and McCoord, A. B.: J. Pediat. **16**:139, 1940.
36. Greaves, J. D., and Schmidt, C. L. A.: Am. J. Physiol. **111**:492, 1935.
37. Greaves, J. D., and Schmidt, C. L. A.: Am. J. Physiol. **111**:502, 1935.
38. Greaves, J. D., and Schmidt, C. L. A.: Am. J. Physiol. **116**:456, 1936.

composed of at least two different substances; (1) an alcohol-soluble component with vitamin A fluorescence and (2) a non-alcohol-soluble red fluorescent one. This supports the conjecture [39] that lipofuscin has a pigment nucleus (originating from protein decay) and a lipoid shell.

ADRENAL GLANDS

The adrenal glands of 208 persons were examined for vitamin A by fluorescence microscopy.

Distribution in Normal Adrenals.—In the adrenals of adults, vitamin A fluorescence was found primarily in the fascicular layer of the cortex and to a minor degree in the glomerular layer (fig. 3 *A*). Only in 3 cases was more vitamin A fluorescence encountered in the glomerular than in the fascicular layer. The medium-sized lipoid droplets in the cytoplasm of the epithelial cells imparted the vitamin A fluorescence, which was less vivid and faded slower than that of the liver. The nucleus was free of vitamin A fluorescence. The vitamin A–carrying lipoid droplets were stained by phosphin 3R. Sections stained with methylene blue showed that in normal adults most of the lipoid droplets elicited the fluorescence.

Double refraction and vitamin A fluorescence were often concomitant and at times were found in a similar distribution. There was, however, no direct connection. In 12 cases relatively more vitamin A fluorescence than double refraction was observed, whereas in 10 cases the contrary was seen. The presence of higher amounts of lipoids with double refraction led to a dim brown-green ultraviolet-stable fluorescence, which was either visible immediately, if no vitamin A was present, or later, after the fading of the vitamin A fluorescence.

Generally, after irradiation a faint brown fluorescence of the glomerular and the outer part of the fascicular layer was noted. In the inner part of the fascicular layer, brown-red fluorescent corpuscles of angular size were seen. These were found more abundantly in the epithelium of the reticular layer. They represented lipofuscin,[1] the amount of which was variable. In the absence of lipoid droplets it extended up to the glomerular layer. The brown-red fluorescence was not influenced by treatment with alcohol and only slightly by that with acetone. It did not give the phosphin 3R reaction. No rule could be ascribed concerning the amount of this lipofuscin fluorescence except that in emaciation and in diabetes mellitus relatively high amounts were seen.

The endothelial cells of the capillaries did not reveal vitamin A fluorescence. In the endothelial cells of the reticular layer, irregular-

39. König, P.: Beitr. z. path. Anat. u. z. allg. Path. **75**:181, 1926. Kutschera-Aichbergen, H.: Frankfurt. Ztschr. f. Path. **27**:21, 1922.

shaped granules of a bright yellow, nonfading fluorescence were seen. After treatment with acetone this fluorescence was more striking. These inclusions varied in amount and at times were absent; there was no regular distribution; in cirrhosis the number was reduced. They indicated a resorptive capacity on the part of the endothelial cells.

Between the medulla and the reticular layer, a "central cortex" developed in the second half of embryonic life, due to invagination of the cortex along the vessels. This perivenous "central cortex," com-

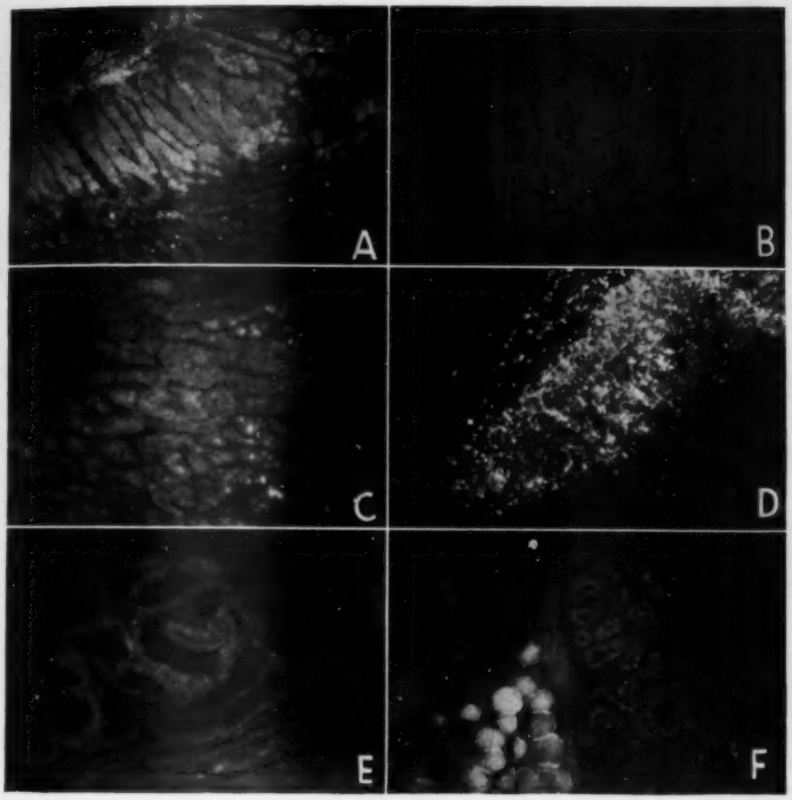

Fig. 3.—Fluorescence photomicrographs: *A*, adrenal of an adult, showing vitamin A fluorescence of the fascicular layer. There are other minute fluorescent inclusions in the capillary endothelial cells of the reticular layer. *B*, adrenal of an infant, showing no vitamin A fluorescence. *C*, focal accumulation of vitamin A fluorescence in the adrenal cortex. *D*, corpus luteum of the ovary, showing vitamin A fluorescence of the granulosa lutein and paralutein cells. *E*, chronic nephritis. There is vitamin A fluorescence of dilated convoluted tubules. The fluorescence of medium-sized lipoid droplets is especially strong. *F*, lactating breast. Vitamin A fluorescence (1) of the glandular epithelium, (2) of the secretion in the lumen of the glands and (3) of the fat cells in the stroma.

posed of derivatives of the glomerular and fascicular layer, showed vitamin A fluorescence usually in the part corresponding with the fascicular layer. The medulla failed to reveal any vitamin A fluorescence.

Total Amount of Vitamin A Fluorescence.—Table 3 shows the total amounts of vitamin A fluorescence in the adrenals of 144 adults as estimated from the histologic pictures. The adrenals of adults, like the livers, invariably showed some vitamin A fluorescence. Traces or small amounts were observed in malnutrition (concomitant with emaciating malignant tumors), in acute infectious diseases and in diseases of the liver. In decompensated cirrhosis it was more marked than in compensated cirrhosis.

TABLE 3.—*Estimation of the Amount of Vitamin A Fluorescence in the Adrenal Cortices of Adults, Grouped According to Diseases*

Diagnosis	Cases in Which Cortex Showed Estimated Amount of Fluorescence					Cases with More in Adrenal Than in Liver	Cases with More in Liver Than in Adrenal	Total
	0	±	+	++	+++			
Cerebral disease and acute death	..	..	..	5	5	..	..	10
Heart disease	..	..	3	7	1	..	..	11
Pulmonary disease	..	..	2	4	2	1	..	8
Renal disease	..	2	4	14	4	2	2	24
Abdominal disease	..	1	2	5	..	..	1	8
Tumor without emaciation	..	1	6	5	..	..	..	12
Tumor with emaciation	..	6	2	5	3	6	5	16
Pulmonary tuberculosis	..	2	2	3	..	..	1	7
Blood disease	..	2	1	4	..	..	1	7
Infectious disease	..	6	2	..	..	..	8	8
Diabetes mellitus	..	1	..	..	3	1	..	4
Pregnancy	..	..	1	1	1	1	..	3
Obstructive jaundice	..	2	1	1	..	1	..	4
Acute hepatitis	..	1	..	2	..	1	..	3
Compensated cirrhosis	..	1	3	5	1	4	..	10
Decompensated cirrhosis	..	3	2	3	1	6	..	9

Uniform reduction was observed in infectious diseases, apparently due to a reduction of the lipoid carrier substances. A comparison of the relative amounts in liver and adrenal indicated that the reduction of the vitamin A fluorescence in acute infectious diseases was more pronounced in the adrenal.

In diseases of the liver the reduction in the adrenal was less uniform than in the liver itself. At times the adrenals showed relatively more than the livers.

The reduction of the vitamin A fluorescence in the presence of tumors with emaciation was not necessarily parallel in the liver and adrenal. The adrenal infrequently revealed abundant vitamin A fluorescence.

In diabetes mellitus the adrenals showed high amounts of vitamin A fluorescence and lipoids. Pregnancy or sex variation was insignificant in connection with the fluorescence microscopic picture in the adrenal.

Development of the Adrenals.—The influence of age was remarkable. The human adrenals are known to undergo extensive changes in early postnatal life.[40]

Parts of the fetal adrenal disappear, and at the tenth year the organ is as large as it was at birth. The hypertrophic primary reticular layer, which represents the chief part of the large adrenal of the newborn infant, degenerates and disappears within the first year. The development of the final reticular layer starts much later. The reconstruction and the development of the folds and lobulations as seen in the adult are completed at the time of puberty. The distribution of the lipoids[41] and the fluorescence microscopic picture change markedly during the development of the adrenal.

Table 4 gives estimations of the vitamin A fluorescence in the adrenals of fetuses, infants and children. In contrast to the liver, the adrenal of the fetus did not show any vitamin A fluorescence (fig. 3 *B*).

TABLE 4.—*Estimation of the Amount of Vitamin A Fluorescence in the Adrenal Cortices of Fetuses, Infants and Children*

Age Group	Cortices Showing Estimated Amount of Fluorescence					Total
	0	±	+	++	+++	
Fetal	3	..	..	..	..	3
Immature newborn	7	..	..	..	..	7
Mature newborn	10	..	..	..	..	10
1 month	5	1	..	..	..	6
2 months	6	3	..	1	..	10
3 months	4	1	..	3	..	8
4-12 months	2	2	..	3	..	7
2-12 years	..	2	3	6	2	13

The first traces appeared in the first month of postnatal life. But during the entire first year only small amounts were found. After this time the storage gradually approached that of the adult.

In the fetus the glomerular and fascicular layer formed a narrow rim and the bulk of the cortex was composed of the primary reticular layer, which Grollman[42] considered as a specific structure, the androgenic tissue. The epithelium of all layers revealed a brown-violet dim fluorescence. In addition the primary reticular layer showed scattered large cells which revealed a striking bluish white fluorescence, often with strong double refraction, and in visible light contained a brown pigment. Isotropic and anisotropic lipoids appear at various times and may be absent after birth.

The epithelial cells of the adrenal of the newborn infant showed a red fluorescence similar to that seen in the liver; it was probably due to the presence of

40. Elliot, T. R., and Armour, R. G.: J. Path. & Bact. **15**:481, 1911. Thomas, E.: Beitr. z. path. Anat. u. z. allg. Path. **50**:283, 1911. Lewis, R. W., and Pappenheimer, A. M.: J. M. Research **34**:81, 1916.

41. Landau, M.: Die Nebennierenrinde, Jena, Gustav Fischer, 1915. Yasukawa, Y: Endocrinology **14**:161, 1934.

42. Grollman, A.: The Adrenals, Baltimore, Williams & Wilkins Company, 1936.

bilirubin (icterus neonatorum). The anisotropic lipoids increased in amount during the first months of postnatal life. From the third month on they were present in relatively the same amount as in adults.

Simultaneously with the appearance of vitamin A fluorescence in the glomerular and fascicular layers, at times the large scattered cells of the reticular layer imparted it. Up to the ninth month not all the lipoid droplets, demonstrable by phosphin 3R, contained vitamin A. After this period the vitamin A fluorescence under normal conditions followed closely the distribution of the lipoids. When the primary reticular layer was gone, the vitamin A–containing cords extended through the whole cortex, up to the medulla. In the subsequent course the folds and lobulations appeared and the vitamin A fluorescence was visible in a central cortex. In children the glomerular layer was usually rich in vitamin A. The same picture was seen in the corresponding area of the central cortex.

The scattered cells in the primary reticular layer disappeared usually after the eighth month. Rarely were they seen up to the seventh year. They probably represented degenerating cells and the last remnants of the reticular layer.

From the third month up to the end of the first year fluorescent inclusions in the endothelial cells were seen. Morphologically these were similar to those of the adult. Apparently they played a role in the resorption of the primary reticular layer. From the second to the eleventh year they were not seen. Afterward the distribution was similar to that of the adult. They may, however, be absent at all ages.

The brown-red fluorescence of lipofuscin was not seen before the fourth month and infrequently up to the third year. Its appearance indicated the development of the final reticular layer. Greater amounts were seen after the eighth year. A marked increase was evident up to the thirtieth year.

No correlation between sex and the fluorescent tissue constituents could be drawn.

Distribution in Pathologic Adrenals.—Many variations of the distribution of the vitamin A fluorescence occurred under pathologic conditions. They usually run parallel with the variations of the lipoid distribution. Heterogeneous distribution of lipoids caused an irregular distribution of vitamin A. If the cortex grossly revealed mottling, the vitamin A fluorescence was present in the yellow areas. If the brown layer extended to the capsule, little vitamin A fluorescence was seen. The gross cut surface was an index for the amount of vitamin A fluorescence present.

Microscopically, the irregular distribution was due either to focal depletion or to focal accumulation of the fluorescence (fig. 3 *C*). These foci represented groups of epithelial cords, single groups of cells or scattered cells. The irregularity was more prominent in the glomerular layer and in the "central cortex." In acute infectious diseases, such as diphtheria, the vitamin A fluorescence disappeared with depletion of the lipoids.

If high amounts of lipoids and vitamin A were present, the vitamin A fluorescence extended through the entire cortex, and the red lipofuscin fluorescence was visible only after irradiation with ultraviolet rays.

Cortical adenoma or areas of adenomatous hypertrophy were rich in vitamin A fluorescence and in anisotropic lipoids; the adjacent adrenal tissue usually contained small amounts, especially in areas compressed by the adenoma. Yellow fluorescent inclusions in the capillary endothelium occasionally occurred and were characteristic for the normal reticular layer. Lipofuscin was seen in circumscribed areas. In one case the areas of adenomatous hypertrophy revealed no vitamin A fluorescence but contained considerable lipofuscin; the surrounding adrenal tissue showed vitamin A fluorescence. In this case the adenomatous area originated from the reticular layer. Accessory adrenals showed a distribution of vitamin A similar to that of the adrenal proper. In pregnancy the hyperplastic glomerular layer was rich in vitamin A.

In jaundice the epithelium of the adrenal imparted a green fluorescence, probably due to the presence of bilirubin. In 2 cases of amyloidosis the areas involved did not show vitamin A fluorescence, although phosphin 3R staining revealed many fine lipoid droplets. In a case of infarction of the adrenals vitamin A fluorescence was considerably reduced.

The vitamin A fluorescence of the fat droplets surrounding the adrenals was not directly related to the vitamin A content of the adrenals.

Comment.—The adrenals are an important storage place for various lipoids. The double refraction of some of the lipoids was originally considered as caused by cholesterol esters,[43] whereas now the specificity of the microchemical reactions is doubted. One of the substances in the lipoid mixture is that giving the vitamin A fluorescence. Apparently, it is related to the yellow color seen at the gross cut surface of the organ. In experiments on rats evidence was offered to show that the characteristic fluorescence in the adrenal cortex was due to vitamin A. It is reasonable to conclude that the fluorescence in human adrenals is caused by vitamin A also, but because of the relatively slow fading we cannot exclude the thought that some of it may be due to the provitamin carotene. Carotene yields, in relatively high concentration, a green fading fluorescence, less brilliant and fading slower than that of vitamin A.[6] The vitamin A fluorescence is not directly related to the double refraction. Both vitamin A and the anisotropic lipoids are dissolved in the same medium.

While in fetuses and infants the adrenal lipoids are free of vitamin A fluorescence, later most lipoids, demonstrable with phosphin 3R, impart the fluorescence. The reduction of the fluorescence in infectious diseases is thus a consequence of the well known depletion of lipoids in these diseases.[44] This parallelism is not so striking in the disappear-

43. Kawamura, R.: Die Cholesterinesterverfettung (Cholesterinsteatose), Jena, Gustav Fischer, 1911.

44. (*a*) Elliot, T. R.: Quart. J. Med. **8**:47, 1914. (*b*) Weltmann, O.: Beitr. z. path. Anat. u. z. allg. Path. **56**:278, 1913.

ance of vitamin A fluorescence during malnutrition or in diseases of the liver. The lipoids are not reduced during malnutrition.[44a]

Bennett [45] showed in cats that chemical substances with reactions similar to those of the biologically active cortical sterones are found in the outer zone of the fascicular layer. It is remarkable that the same area usually yields the strongest vitamin A fluorescence.

Fluorescence microscopy, which permits a clear differentiation of the pigments of the adrenal, yields a characteristic picture of the development of the organ and helps in the analysis of cortical adenomatous hypertrophy. The primary reticular layer—the androgenic tissue—is free of vitamin A, although the endocrine structures of the gonads imparted it as discussed in the following section.

TESTICLE

The testicles of 22 persons were examined. Up to puberty no striking fluorescent constituents were visible except for the connective tissue fibers. Afterward different fluorescent inclusions appeared, which partly revealed the green fading fluorescence of vitamin A.

Leydig Cells.—The interstitial cells of Leydig revealed brown fluorescent granules, brown in visible light, after puberty.[1] The brown fluorescence disappeared partly after treatment with alcohol and nearly completely after treatment with acetone. The fluorescence was often reduced or absent at higher ages. The Leydig cells showed strong double refraction and gave a phosphin 3R reaction. In most Leydig cells, a strong, slowly fading green fluorescence, which masked the brown fluorescence, appeared before irradiation. The amount of the vitamin A fluorescence varied. In older persons and sometimes in persons suffering from general exhaustion the vitamin A fluorescence of the Leydig cells was absent—apparently before the disappearance of the brown fluorescence. After acetone treatment striking yellow-brown fluorescent inclusions were visible in the endothelial cells of the capillaries.

Seminiferous Tubules.—In the tubular cells varied fluorescent inclusions appeared after puberty. The Sertoli cells contained high amounts of small and large lipoid droplets that showed a green-yellow fluorescence. After irradiation the green tinge, apparently due to the presence of vitamin A, disappeared and a brown fluorescence remained. The latter disappeared after treatment with alcohol and acetone. The inclusions gave the phosphin 3R reaction and moderate double refraction. The lipoid droplets disappeared with increase in age.[46] Smaller amounts of these fluorescent lipoids were seen in the spermatogenic cells, and

45. Bennett, H. S.: Am. J. Anat. **67**:151, 1940.

46. Berberich, J., and Jaffé, R.: Frankfurt. Ztschr. f. Path. **27**:395, 1922.

again traces of the vitamin A fluorescence were noted. Thus, the fluorescence could be traced in decreasing amounts through all layers of the tubules up to the lumen.

In the spermatogenic and Sertoli cells angular-shaped corpuscles of brown-red fluorescence were often visible. These were insoluble in alcohol and partly soluble in acetone. These corpuscles, representing lipofuscin, revealed a green tinge, which quickly disappeared on irradiation. The lipofuscin increased in amount with increase in age.

With involution of the testicles, the blue fluorescent basement membrane proliferated and the pigments with vitamin A fluorescence gradually disappeared. Varying-sized granules of striking yellow ultraviolet-stable fluorescence appeared in all layers of the tubules. They coalesced and filled nearly the entire lumen. These granules which were not seen in the testicles of younger men were not soluble in alcohol or acetone.

Traces of fading green fluorescence were usually seen in the tubular cells when the yellow-brown lipoid fluorescence or the angular granules of lipofuscin fluorescence were observed. No relation between this vitamin A fluorescence and that of the Leydig cells, the vitamin A depots in other organs or the general nutritional condition was obvious.

In the epididymis no vitamin A fluorescence was found, although lipofuscin was seen.

Comment.—The testicles, rich in lipoids and pigments, reveal four different fluorescent details: (1) the brown pigment in the cells of Leydig (its solubility in acetone does not support the view assumed by some investigators [47] that this pigment is of "wear and tear" nature; the vitamin A fluorescence suggests an endocrine activity); (2) the lipoid droplets in the tubular cells; (3) the lipofuscin in the tubular cells; (4) the irregular yellow confluent granules associated with involution of the testicles. The physiologic significance of these details, especially of the lipoid droplets in the tubular cells and their connection with endocrine activity, is a promising problem for future work.

Vitamin A fluorescence is present in the first three of these fluorescent bodies. Whether the slowly fading fluorescence of the Leydig cells is due to carotene should be considered.

The vitamin A fluorescence of the testicles revealed a definite relation to the activity of the organ. It appears with maturity and disappears with involution, the latter being indicated by (1) the absence of spermatogenesis, (2) the disappearance of the fluorescent lipoids, (3) the proliferation of the basement membrane and (4) the appearance of the yellow fluorescent granules.

47. Priesel, A.: Virchows Arch. f. path. Anat. **249**:246, 1924. Stieve, H.: Männliche Genitalorgane, in von Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1930, vol. 7, pt. 2.

OVARY

Ovaries of 26 persons representing different age groups were examined. Great variations in the microscopic picture were encountered, depending on the menstrual cycle. In all age groups, a strong blue fluorescence was imparted by the fibrous stroma and by the thick walls of the arteries, which in addition revealed the gray fluorescence of the elastic fibers. The corpora albicantia and candicantia revealed a strong blue fluorescence. The immature follicles did not reveal a characteristic fluorescence.

In the ovaries of newborn girls a few irregular-shaped granules of an ultraviolet-stable brown fluorescence were seen.

Corpus Luteum.—In the mature organ the corpus luteum imparted vitamin A fluorescence (fig. 3 *D*). Four different layers, the development and size of which depended on the menstrual cycle, were apparent. In the center, the nucleus revealed a moderate blue fluorescence, due to the fine connective tissue and fibrin fibers. A black area of hemorrhage followed—iron interferes with fluorescence. The next layer was the folded zone of the granulosa lutein cells. These cells usually revealed vitamin A fluorescence either localized in medium-sized droplets on the edges of the cells or fainter, diffusely dispersed in the cytoplasm. After fading of the green tinge, these droplets stood out faint brown. In addition the cytoplasm of the cells often had a nonfading green or brown fluorescence and in places contained irregular-shaped nonfading green granules. Some of the granulosa lutein cells, not unlike the others in visible light, failed to show droplets with vitamin A fluorescence. The phosphin 3R reaction varied, apparently with the menstrual cycle; in some cases little phosphin 3R reaction was given by the granulosa lutein cells, whereas in others more lipoids were demonstrable than would be expected from the vitamin A fluorescence. Usually slight double refraction was present. Alcohol or acetone removed the fluorescence of the granulosa cells except for some remnants of the brown fluorescence. The last layer, the theca lutein or paralutein cells [48] in the lobulations of the granulosa cell layer, often had larger droplets, which imparted a fainter, though slower fading, vitamin A fluorescence than the granulosa lutein cells. The droplets usually gave the phosphin 3R reaction and revealed in places definite double refraction. The development of these cells was more variable than that of the granulosa lutein cells. In the transition to the corpus albicans or candicans the green and brown fluorescence of the corpus luteum gradually disappeared, the theca cells being more prominent during the later stage.

48. Novak, E.: Gynecological and Obstetrical Pathology with Clinical and Endocrine Relations, Philadelphia, W. B. Saunders Company, 1940.

Corpus Atreticum.—The centers of the corpora atretica yielded a strong blue fluorescence. Large droplets within the proliferated theca cells around the corpora atretica revealed strong vitamin A fluorescence, which faded relatively slowly. After this fading a definite brown fluorescence remained and was also seen after treatment of the sections with alcohol. Acetone removed it incompletely. Phosphin 3R produced a very distinct fluorescence of these cells. Their double refraction was also striking—the strongest seen in the ovary.

The vitamin A fluorescence of the corpus atreticum depends on its developmental stage and the menstrual cycle. Often during the full development of the corpus luteum the corpora atretica in the same or in the contralateral ovary yielded strong fluorescence. In the dissolution of the corpus atreticum the characteristic starlike distribution of the proliferated theca cells disappeared and the cells were scattered over the cortical stroma, isolated or in small groups. They gradually lost their vitamin A fluorescence. Sometimes a part of the same cell showed the fluorescence while another part revealed the brown fluorescence which was seen in the entire cell after the fading of the vitamin A fluorescence or after the removal of the latter by alcohol. Some cells, in a group, revealed the vitamin A fluorescence while others fluoresced brown immediately. Finally all cells had lost the vitamin A fluorescence simultaneously with the phosphin 3R reaction, whereas the double refraction remained longer.

The scattered cells were hardly recognizable as former theca lutein cells. The brown fluorescent substance appeared in visible light less distinctly as brown pigment and was seen to a lesser degree in the theca cells of the intact corpora atretica. In visible light there was no difference between these scattered theca lutein cells containing vitamin A and those free of it. In certain stages of the menstrual cycle the scattered theca lutein cells seemed to acquire vitamin A fluorescence again, coinciding with the reappearance of isotropic and anisotropic lipoids.

Stroma.—The cortical stroma of the ovary, which after the climacterium did not reveal lipoids with vitamin A fluorescence, showed such a finding frequently during the regenerative stage. In places the fibrous stroma appeared to be sprinkled with fine droplets that revealed a quickly fading vitamin A fluorescence. With phosphin 3R these anisotropic droplets were seen as pinpoints. Their distribution was not uniform, their number being increased around luteinized corpora atretica and also around corpora lutea. In some stages of the menstrual cycle vitamin A fluorescence was seen in various amounts in almost the whole cortical stroma. In other stages the greatest part of the stroma was free of the fluorescence. Between different corpora atretica could be seen bridges of the vitamin A–rich stroma. In all stages the stroma contained irregularly formed brown, red or yellow fluorescent bodies. Their fluorescence was partly removed by lipoid solvents.

Follicular Cysts.—The walls of follicular cysts showed vitamin A fluorescence in the granulosa and theca cell layers, if both were developed. In the granulosa cell layer the fluorescence appeared in fine droplets; in the theca cell layer in larger droplets. The droplets did not always give the phosphin 3R reaction, and the double refraction was usually indistinct.

In the ovary of an 11 year old girl, who succumbed to a brain tumor, there were encountered many follicular cysts, the walls of which showed extensive vitamin A fluorescence in both layers. In places a coalescence of the droplets in the granulosa cells, with strong vitamin A fluorescence of the larger drops, was seen.

Pregnancy.—One ovary from a pregnant woman was examined. The woman had died from pernicious vomiting; the liver and adrenal showed traces of vitamin A fluorescence. The granulosa cell layer of the corpus luteum showed a few droplets with vitamin A fluorescence and a diffuse green fluorescence. No lipoids were demonstrable with phosphin 3R. The walls of some follicular cysts showed small amounts of vitamin A fluorescence.

Climacterium.—After the climacterium neither vitamin A fluorescence nor phosphin 3R reaction nor double refraction was demonstrable. The scattered remnants of the theca lutein cells of the corpora atretica were not seen, and only a few brown or red inclusions were encountered in the fibrous ovary.

Comment.—Escher [49] first described the presence of carotene in the corpus luteum, in 1913. The yellow pigment of the corpus luteum is supposedly due to carotene. The fluorescence of carotene fades much slower and is much weaker than that of vitamin A. The fluorescence in the ovary may, therefore, be due either to high amounts of the provitamin carotene or to vitamin A itself. The fluorescence of the granulosa cells and of the stroma is too bright and fades too quickly to be due to carotene; that of the theca cells, especially of those around the corpora atretica, is possibly due to carotene. The vitamin A fluorescence cannot be correlated with the yellow color in the corpus luteum. According to Walthard,[50] sulfuric acid produces a green to blue color of the theca lutein cells of the atretic follicles. This reaction is not specific for carotene since it corresponds to the Drummond reaction of vitamin A.[51]

The role of vitamin A in the ovary is not established. It is supposedly related to reproduction.[52] The vitamin A fluorescence of the ovary was not found related to the nutritional stage of the organism, in

49. Escher, H. H.: Ztschr. f. physiol. Chem. **83**:198, 1913; Arch. f. Gynäk. **119**:1, 1923.

50. Walthard, K. M.: Ztschr. f. Geburtsh. u. Gynäk. **86**:74, 1923.

51. Fearon, W. R.: Biochem. J. **19**:888, 1925.

52. Evans, H. M.: J. Biol. Chem. **77**:651, 1928. Mason, K. E., and Ellison, E. T.: J. Nutrition **9**:735, 1935; **10**:1, 1935.

contrast to that of the liver and that of the adrenal. The lipoids also are independent of the nutritional stage.[53] The morphologic picture of the fat storage varies with the menstrual cycle and the development of the corpus luteum. Although some [54] assume that the hormonal activity is strongest when the most lipoids are seen, most investigators agree that lipoids are morphologically demonstrable only during the involution of the corpus luteum.[55] The anisotropic lipoids seem to be more related to hormonal activity.[56] The chemical assay of lipoids is more significant than the histologic picture: The same lipoid content is found in the full-developed and in the degenerating corpus luteum.[57] Phospholipoids were found in the active stage of the corpus luteum, in contrast to the prevalence of cholesterol and fatty acids during involution.[58]

Vitamin A fluorescence is demonstrable in the fully developed corpus luteum and sometimes out of proportion to the histologically demonstrable lipoids. The vitamin A fluorescence seems to be at a peak in this stage. The same seems to be true for the stroma and the theca lutein cells of the corpora atretica. Vitamin A fluorescence appears to be a morphologic sign of luteinization; it is characteristic of an active stroma, which in visible light is not different from the common fibrous stroma. Investigations are in progress to examine this hypothesis. The lipoids, whether histologically demonstrable or not, are to be considered as carriers not only of hormones [59] but also of vitamin A.

The fluorescence microscopic picture of the ovary, which was described briefly as early as 1924,[60] may be helpful in the histologic analysis of the organ through utilization of the vitamin A fluorescence as a morphologic characteristic. Three different structures show vitamin A fluorescence in different distributions: the granulosa lutein cells, the theca lutein cells and the active stroma. According to Fischel's theory,[61] supported by Meyer [62] and Schiller,[63] all these structures derive from the same mesenchymatous tissue, which has a potentiality for vitamin A storage and for hormonal activity.

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53. Preissecker, E.: Zentralbl. f. Gynäk. **52**:2740, 1928.
54. Berberich, J., and Jaffé, R.: Ztschr. f. ang. Anat. **10**:1, 1925.
55. Meyer, R.: Arch. f. Gynäk. **93**:355, 1911. Marcotty, A.: ibid. **103**:63, 1914.
56. Geist, G. H., and Gaines, J. A.: Am. J. Obst. & Gynec. **35**:39, 1938.
57. Kaufmann, C., and Raeth, K.: Arch. f. Gynäk. **130**:128, 1927.
58. Traut, H. F.; Kuder, A., and Cadden, J. F.: Am. J. Obst. & Gynec. **38**: 798, 1939.
59. Pratt, J. P.: Arch. Path. **19**:380, 1935. Zondek, B., and Aschheim, S.: Arch. f. Gynäk. **127**:250, 1926.
60. Policard, A.: Compt. rend. Acad. d. sc. **179**:1287, 1924.
61. Fischel, A.: Ztschr. f. Anat. u. Entwcklngsgesch. **92**:34, 1930.
62. Meyer, R.: Am. J. Obst. & Gynec. **28**:302, 1934.
63. Schiller, W.: Surg., Gynec. & Obst. **70**:773, 1940.

The vitamin A fluorescence of the theca lutein cells with their typical proliferation in pregnancy or during cyclic luteinization is characteristic. It is more prominent than the double refraction [64] which in the past has led to confusion with pseudoxanthoma cells. The vitamin A fluorescence permits a study of the cells during the dissolution of the corpus atreticum. Terminally these cells are seen in groups [65] or isolated in the ovarian stroma, still imparting vitamin A fluorescence. These scattered theca lutein cells finally lose their vitamin A fluorescence. The brown fluorescent cells remain and reveal a brown pigment in visible light also. With the climacterium, these "brown" cells disappear. A more extensive study of these cells and their histogenesis will be published at a further date.

If the vitamin A fluorescence may be considered as indicating hormonal activity, these scattered theca lutein cells retain for a certain time the same hormonal activity which the theca cells around the corpora atretica possess.[66] The "brown" cells may reacquire the fluorescence for some time during cyclic luteinization. The vitamin A fluorescence supports the assumption that there exists an interstitial gland, especially since the interstitial cell cords of the rat's ovary show the same slow-fading fluorescence and the same double refraction imparted by large droplets as the theca lutein cells around the corpora atretica and those scattered in the cortical stroma.

FAT CELLS

The fat cells in adipose tissue and those scattered in the stroma of various organs imparted a vitamin fluorescence, which was dim and faded relatively slowly (fig. 3 *F*). Although great variations were encountered, the vitamin A fluorescence never was high. Generally, the smaller the fat droplets the stronger was their fluorescence. The smallest fat cells sometimes revealed fluorescence equal to that of an epithelial cell of the adrenal cortex.

There were great variations of the vitamin A fluorescence between the various fat stores of the body or between the scattered fat cells, e. g., in the submucous layer of the intestine and in the subcutaneous tissue. There was no relation between the vitamin A fluorescence of the fat depots and that of the liver or that of the adrenal.

A dependence on the nutritional stage in general was apparent. In emaciation the vitamin A fluorescence was reduced. After the fading of the vitamin A fluorescence, a darker green, ultraviolet-stable fluorescence remained, due to the fat pigments. Its intensity varied in

64. Adachi, S.: Ztschr. f. Geburtsh. u. Gynäk. **76**:125, 1914.
65. Keller, R.: Beitr. z. Geburtsh. u. Gynäk. **19**:13, 1914.
66. Corner, G. W.: Physiol. Rev. **18**:154, 1938.

the different fat depots of the same subject. This green fluorescence over which the vitamin A fluorescence was superposed, at times, caused an overestimation of the vitamin A fluorescence in the fat cells.

In necrotic fat tissue, as in pancreatic fat necrosis, there was no vitamin A fluorescence. Instead, there was a striking ultraviolet-stable yellow fluorescence. Fatty necroses, like those in abscesses or tumors, yielded no vitamin A fluorescence, although many other fluorescence phenomena were found. The same was true for the anisotropic lipoids in pseudoxanthoma cells.

Comment.—Chemical analyses have revealed that fat tissue is a storage place for vitamin A. Although the vitamin A content of the individual fat cell is relatively low, the great mass of fat cells in the whole body makes the latter the most important vitamin A depot second to the liver.

MAMMARY GLAND

Nonlactating Gland.—The glandular tissue of the nonlactating breast showed no vitamin A fluorescence. Only the fluorescence of the adjacent fat cells was visible.

Lactating Gland.—The glands of the lactating breast were very rich in vitamin A fluorescence. The epithelial cells of the alveoli contained small to medium-sized lipoid droplets, which imparted a strong vitamin A fluorescence (fig. 3 *F*). After fading of the green tinge, a strong blue fluorescence remained. Sections stained with methylene blue revealed that all lipoid droplets imparted vitamin A fluorescence. Toward the internal poles of the cells the fluorescent lipoid droplets accumulated and became larger. Inside the lumens of the alveoli and of the alveolar and excretory ducts large lipoid droplets were seen. These also yielded strong vitamin A fluorescence.

The histologic picture of the lactating breast corresponds with the high vitamin A content of the milk and especially with that of the colostrum.[67]

KIDNEY

The kidneys of 105 persons were examined. These covered different age groups and included pathologic organs.

Pigments.—Various spontaneously fluorescent non-lipoid-soluble details were visible. Brown fluorescent granules were seen in the epithelium of the Henle loops and of the distal convoluted tubules and often were localized at the base of the epithelial cells. Sometimes a tubular lumen contained a brown fluorescent mass, while the common hyaline and granulated casts revealed white fluorescence. The brown bodies were much increased in number in the newborn state and in cirrhosis and especially in jaundice. At times the brown fluorescence was restricted to

67. van Eekelen, M., and de Haas, J. H.: Acta brev. Neerland. **4**:52, 1934. Semb, J.; Baumann, C. A., and Steenbock, H.: J. Biol. Chem. **107**:697, 1934.

isolated nephrons. In jaundice a green diffuse fluorescence, especially of the convoluted tubules, was also noted. In other conditions, yellow and red fluorescent granules were encountered. In the glomeruli fine white granules were seen accumulated at the vascular pole. In scar tissue bizarre-shaped golden red inclusions occurred. In pathologic kidneys the various fluorescent details were increased in number and produced a bizarre picture. In contracted kidneys the proliferated elastica increased the distortion.

Some of the pigments are probably derivatives of hemoglobin, which according to Lubarsch [68] are common in the kidney.

Lipoids.—Normal kidneys did not show vitamin A fluorescence. In many normal kidneys lipoids were stained by phosphin 3R (not always by sudan III). Of 59 kidneys without vitamin A fluorescence, 13 were proved to be without lipoids. These 13 organs included pathologic kidneys also, whereas among the fat-carrying kidneys normal organs and kidneys of newborn infants were encountered. Usually more lipoids were found in pathologic kidneys. Of the 46

TABLE 5.—*Estimation of the Amount of Vitamin A Fluorescence in Kidneys Revealing Vitamin A Fluorescence*

Diagnosis	Cases	Estimated Amount of Renal Fluorescence
Acute nephritis	1	+
Subacute nephritis	2	++ to +++
Chronic nephritis	5	++ to +++
Pregnancy kidney with nephrotic picture	2	+ to ++
Amyloidosis	2	+
Benign nephrosclerosis	4	± to +
Malignant nephrosclerosis	1	+
Infarcts of the kidney	1	++
Exophthalmic goiter with glomerular irritation	1	+
Pemphigus	1	+
Typhoid fever	1	+
Myelogenous leukemia	1	±

giving positive results, 21 revealed fat storage only in the medulla; 1, in the cortex, and 24, in both, with prevalence in the cortex.

In the cortex, usually fine droplets were seen at the base of the cells of the proximal and distal convoluted tubules. Only small amounts were found in the stroma, and in 3 cases fine droplets were seen in the glomeruli. In the medulla, infrequently similar droplets were noted at the base of the cells of the Henle loops. The bulk was encountered in the interstitium. The thickened connective tissue of the medullary stroma at times showed an indistinct phosphin 3R reaction. The fat storage on the whole revealed a nephron-bound distribution.

Vitamin A Fluorescence.—In all kidneys in which vitamin A fluorescence was demonstrated some kind of renal damage was encountered (table 5). The highest amounts were found in those showing inflammatory conditions and moderate amounts in those

68. Lubarsch, O.: Ueber die pathologischen Ablagerungen, Speicherungen und Ausscheidungen in den Nieren (Lipoid-, Glykogen-, Pigment-, Kalk- und Salzablagerungen), in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1925, vol. 6, pt. 1.

showing degenerative conditions, whereas those with vascular diseases revealed only traces. In 8 cases of benign and 2 of malignant nephrosclerosis, 1 case of pyelonephritis and 1 case of acute nephritis the kidneys did not show any vitamin A fluorescence.

The fluorescence was usually encountered in the proximal convoluted tubules, in fine droplets at the base of the epithelial cells. In places a continuous band of fine, quickly fading droplets lined the base of the cells and produced a starlike picture at the junction of three cross-sectional tubules. In the vicinity of vitamin A–carrying tubular cells, the stroma histiocytes contained fine droplets with vitamin A fluorescence, which gave the stroma a sprinkled appearance. The vitamin A fluorescence was usually restricted to certain nephrons and the adjacent stroma. Rarely did the stroma alone reveal vitamin A fluorescence. In nephritis, with more advanced damage large drops imparted vitamin A fluorescence, and these were localized in the vacuolated epithelial cells of the hypertrophic tubules (fig. 3 *E*). In 4 cases vitamin A fluorescence was seen in the glomeruli; in 1 of these it was noted in the proliferated epithelium of Bowman's capsule; in another it was seen in the fused glomerular loops.

In a case of subacute glomerulonephritis concentrated sucrose solution had been given intravenously before death and the characteristic renal changes [69] developed. The hydropic degenerating tubular epithelium was free of vitamin A, the cells containing only a few vacuoles, but the surrounding stroma imparted vitamin A fluorescence.

In some cases vitamin A fluorescence was seen in the Henle loops and in the distal convoluted tubules. In all but 1 case it was present in fine droplets and usually in a nephron-bound distribution. The medullary stroma showed fine droplets with vitamin A fluorescence, which accumulated at the corticomedullary boundary.

In all kidneys with vitamin A fluorescence high amounts of lipoids were demonstrable by phosphin 3R and usually more than showed vitamin A fluorescence. The sudan III reaction was at times negative when traces of vitamin A fluorescence were present. If only the cortex or the medulla revealed vitamin A fluorescence, much lipoid material was demonstrable in both. In 4 cases the vitamin A fluorescence ran parallel with the double refraction, but in the majority of the cases there was no direct relation between vitamin A fluorescence and double refraction.

In the walls of abscesses lipoids were seen to impart a yellow ultraviolet-stable fluorescence but showed no vitamin A fluorescence.

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69. Anderson, W. A. D., and Bethea, W. R., Jr.: J. A. M. A. **114**:1983, 1940. Lindberg, H. A.; Wald, M. H., and Barker, M. H.: Arch. Int. Med. **63**: 907, 1939.

Comment.—There is no agreement in the literature as to whether the normal kidney reveals fat on histologic examination. While some [70a] observers have considered a fat reaction a pathologic sign, others [70b] have described fat storage in the normal kidney, at least in some tubules. As to the chemistry of the fats, different opinions have been reported.[71] The double refractive lipoids are supposedly cholesterol esters or myelin and protargon.[72]

The kidney showing vitamin A is a pathologic organ, in contrast to the other organs discussed. The storage is independent of the nutritional stage. Although the presence of lipoids with double refraction is also a pathologic sign, there is no direct relation between this and vitamin A fluorescence; both are found in epithelium and stroma.

The reason for the vitamin A storage in the kidney is probably the same as for the pathologic storage of isotropic and anisotropic lipoids. The older theories are now discarded: (1) that these fats originate from local decay of the cells; (2) that the lipoids as normal metabolites remain in the cells because of a toxic damage of the cells; (3) that damaged cells of the kidney absorb the lipoids from the blood stream; (4) that the presence of lipoids is the result of excretion of lipoids by the renal tubules. All these theories assume that the stroma absorbs lipoids from degenerating epithelial cells of the tubules.

According to the recent theories of lipoid nephrosis,[73] lipoids are filtered through the glomerular loops, in contrast to the norm. Within the tubules an attempt at reabsorption of the lipoids together with the normal water reabsorption takes place. This causes lipoid storage within the tubules and, in the further course of the reabsorption, within the interstitium. The presence of the isotropic and anisotropic lipoids, therefore, is the result of a pathologic permeability of the glomerular loops and is not due to a primary damage of the tubular epithelium.

The histologic demonstration of vitamin A in the kidney together with the known findings on excretion of vitamin A supports the foregoing theory. Vitamin A is not found in human urine under normal conditions [74] but is found under pathologic conditions, such as damage

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70. (*a*) Fischer, W.: Beitr. z. path. Anat. u. z. allg. Path. **49**:34, 1910. (*b*) Maximow, A. A., and Bloom, W.: Textbook of Histology, ed. 2, Philadelphia, W. B. Saunders Company, 1939.

71. Segawa, M.: Beitr. z. path. Anat. u. z. allg. Path. **58**:1, 1914. Bell, E. T.: J. Path. & Bact. **19**:105, 1914.

72. Stoerk, O.: Sitzungsb. d. k. Akad. d. Wissensch. Math.-naturw. Kl. **115**: 31, 1906.

73. Randerath, E.: Ergebn. d. allg. Path. u. path. Anat. **32**:91, 1937. Bell, E. T.: Am. J. Path. **14**:691, 1938.

74. von Drigalski, W.: Ztschr. f. Vitaminforsch. **3**:37, 1934. Schneider, E., and Weigand, H.: Klin. Wchnschr. **16**:441, 1937.

of the kidney and infections.[75] Vitamin A appears in the urine during chronic nephritis; its presence is usually associated with high albuminuria but is not directly related to a high level of vitamin A in the blood.[76]

The presence of vitamin A in the urine can be considered as a sign of changed permeability of the glomerular loops and may be a more sensitive indicator of these changes than the demonstration of lipoids with double refraction. The vitamin A fluorescence in the renal tissue, therefore, is a sign of changed permeability of the glomerular loops as well. The lipoid nephrosis as such is a more advanced stage of this process.

In some animals vitamin A fluorescence of the kidney is a normal occurrence. Especially is it encountered in dogs, where it is imparted by small and large isotrophic lipoid droplets within the tubular epithelium.

MISCELLANEOUS ORGANS

Nearly all human organs showed some fluorescence, and often it was due to lipoids. Except for the discussed sites, no vitamin A fluorescence was encountered in the following organs (only the scattered fat cells in the stroma of various organs revealed the vitamin fluorescence):

- Brain
- Pineal body
- Pituitary gland
- Thyroid gland
- Pancreas (brown fluorescent granules in islet[77])
- Spleen (many fluorescent inclusions)
- Lymph nodes
- Tonsils
- Lungs and bronchi
- Heart muscle (red fluorescent lipofuscin inclusions)
- Arteriosclerotic plaques of aorta (double refractive and nonfading fluorescent inclusions)
- Gallbladder
- Epithelium of gastrointestinal tract
- Epidermis (yellow fluorescent granules in epithelium of sweat glands)
- Prostate (yellow fluorescent granules in epithelium of glands)

The myometrium revealed fine granules of a red nonfading fluorescence. The endometrium showed granules of brown fluorescence.

The fluorescence of the human retina has not been studied.

GENERAL COMMENT

Vitamin A fluorescence is a physical property of some body lipoids. There are only a few examples in which vitamin A fluorescence was imparted by tissue constituents which did not give fat reactions either

75. Boller, R.; Brunner, O., and Brodaty, E.: Wien. Arch. f. inn. Med. **31**:1, 1937.

76. Hedberg, J., and Lindquist, T.: Acta med. Scandinav., 1939, supp. 90, p. 231.

77. Grafflin, A. L.: Anat. Rec. **78**:207, 1940.

with the common fat stains or at least with phosphin 3R. (Possibly the fluorescence was here produced by carotene.) In liver cells or in granulosa lutein cells of the ovary during certain phases of the menstrual cycle chemical analysis reveals lipoids which do not stain with the ordinary fat stains. The vitamin A fluorescence is independent of the double refraction of the lipoids.

Not all lipoids give vitamin A fluorescence. Those which show it reveal great variations in the intensity. This indicates that the presence of vitamin A in the lipoids is caused not by a nonspecific solution of vitamin A in the body lipoids but by a specific affinity of certain lipoids to vitamin A. Fats in areas of necrosis or necrobiosis are free of vitamin A. The diseased liver cell loses its vitamin A, while the lipoids remain. This points to a differentiation of physiologic lipoid storage or infiltration from pathologic lipoid deposition or degeneration.

The histologic demonstration of vitamin A is more advantageous than the chemical analysis in several respects. First, the histologic method is more sensitive if the organ has a heterogeneous distribution of vitamin A. If vitamin A is located only in certain cells of the organ, the chemical analysis does not detect small quantities in the great mass of tissue free of vitamin A. A comparison between the histologic and the chemical method has shown that both are equally sensitive if the organ has a homogeneous distribution, as does the liver.[6] Second, the presence of vitamin A in the fat cells may be mistaken for occurrence of the vitamin in the specific parenchyma. Third, quantitative chemical findings do not reveal the nature of the distribution. The distribution may sometimes be more significant than the amounts; e. g., from a functional point of view, vitamin A in the Kupffer cells of the liver has a significance different from that of vitamin A in the epithelial cells.

Vitamin A fluorescence in human organs may be either physiologic or pathologic. In the first instance amount and distribution depend on two factors: (1) the nutritional stage of the organism and (2) the functional stage of the organ. The nutritional stage influences the amount and distribution of vitamin A in the liver, the adrenal and adipose tissue. These are storage places for vitamin A, the liver being the most important one. The vitamin A of the fat cells is less available for immediate use, as these cells are depleted more slowly. Functional factors, in addition, determine the amount and distribution in the liver and adrenal. In damage of liver cells the storage is reduced; there are great variations in the adrenal, independent of the nutrition. In the testicle, ovary and breast functional factors are decisive. In the breast lactation causes vitamin A fluorescence. Our findings suggest that the vitamin A fluorescence in the gonads is a morphologic sign of endocrine activity, although the relation between sex hormone production and vitamin A is not established as yet. In the kidney the presence of

vitamin A is a pathologic sign and is indicative of disturbed permeability of the glomerular loops.

Many organs do not reveal vitamin A fluorescence, even when lipoids are demonstrable histologically. It should be emphasized that those epithelial structures which, according to Wolbach [78] and others, are the first sites to be affected in vitamin A deficiency do not show vitamin A fluorescence under normal conditions. This is true for epidermis and for epithelium of the gastrointestinal tract, bronchial tree and urinary passages. The same findings are obtained in rats, e. g., absence of vitamin A fluorescence from the cornea, even in hypervitaminotic rats. If the chemical analysis reveals vitamin A in those organs, it is due to storage in fat cells, as in the corium or the submucosa, and not to storage in the specific parenchyma. The absence of vitamin A from the structures first involved in vitamin A deficiency suggests a distant effect of vitamin A in those places.

Vitamin A fluorescence is imparted by both epithelial and mesenchymatous structures. In rats vitamin A fluorescence was found in capillary endothelial cells and connective tissue elements of various organs, such as kidney, lung, epicardium, pleura and meninges. In human organs it was found among mesenchymatous elements only in the Kupffer cells, provided one omits the more specific structures, such as adipose tissue or active stroma of the ovary. It is remarkable that the spleen is free of vitamin A fluorescence in man and in the rat, although the endothelial cells of the spleen play an important role in the reticuloendothelial system.

If one omits a few instances of hepatic damage in newborn and older infants, there was no case in which vitamin A fluorescence was entirely absent. Experiments on rats have shown that the first clinical signs of vitamin A deficiency appear somewhat after the disappearance of the vitamin A fluorescence. None of the human subjects of the present investigation showed clinical signs of vitamin A deficiency. Various nutritional surveys [79] and clinical examinations of dark adaptation [80] have indicated that hypovitaminosis exists in a certain percentage of the population. If these conclusions are correct—the value of the adaptometric methods is still sub judice [81]—one has to assume that in man hypovitaminosis may occur while considerable vitamin A reserves are present in the body. An impaired utilization has to be considered.

78. Wolbach, S. B.: J. A. M. A. **108**:7, 1937.

79. Booher, L. E.: J. A. M. A. **110**:1920, 1938. Stiebeling, H. K., and Phipard, E. F.: Diets of Families of Employed Wage Earners and Clerical Workers in Cities, Circular 507, United States Department of Agriculture, 1939.

80. Jeghers, H.: J. A. M. A. **109**:756, 1937; Ann. Int. Med. **10**:1304, 1937. Sloan, L. L.: Arch. Ophth. **21**:913, 1939.

81. Steininger, G., and Roberts, L. J.: Arch. Int. Med. **64**:1170, 1939. Isaacs, B. L.; Jung, F. T., and Ivy, A. C.: Arch. Ophth. **24**:627, 1940.

According to the histologic and chemical investigations, the danger of vitamin A deficiency exists in newborn and older infants, in patients with diseases of the liver and jaundice, in those with uremic renal diseases and in those with various infections.

Vitamin A fluorescence is important not only from the point of view of the physiology of vitamin A but from that of the characteristic morphology of certain structures. This is demonstrated by some of the problems in the study of which vitamin A fluorescence may be helpful: the differentiation between physiologic and pathologic fat storage; the Kupffer cell–liver cell block; the development of the adrenal; the genesis of lipoid nephrosis. The vitamin A fluorescence of the cells supposedly concerned with sex hormone production or storage (such as the Leydig cells of the testicle or the corpus luteum, corpus atreticum and interstitial gland of the ovary) indicates that vitamin A fluorescence may be used as a morphologic sign of gonadal activity.

SUMMARY AND CONCLUSIONS

The histologic distribution of vitamin A in the human body as studied by means of fluorescence microscopy has been described. The fluorescence microscopic picture in various organs, especially so far as the pigments and lipoids are concerned, has been analyzed. The technic has been described, and additional evidence has been offered to show that the green fading fluorescence which was studied in human organs is due to the presence of vitamin A.

The vitamin A fluorescence is imparted by lipoids of the epithelial and Kupffer cells of the liver, of the epithelial cells of the adrenal cortex, of the tubular and Leydig cells of the testicle, of the granulosa and theca lutein cells and the cortical stroma of the ovary, of the adipose tissue and fat cells, of the glands of the lactating breast and of pathologic kidneys. Great variations occur in the amount and distribution under normal, and yet greater under pathologic, conditions. The fluorescence in the liver and the adrenals is influenced (1) by the age of the subject; (2) that in the liver, the adrenals and fat tissue, by the nutritional stage of the subject, and (3) that in the gonads and the breasts, by the functional stage of the organ. In addition, the fluorescence in the liver is reduced in the damages of the liver cells and that in the adrenals in infections. Vitamin A fluorescence of the kidney indicates pathologic permeability of the glomerular loops.

Independent of its significance in the study of vitamin A metabolism, vitamin A fluorescence as a morphologic characteristic of certain structures is helpful in the investigation of various problems. The significance of many fluorescent details in the adrenals and gonads and the relation of these details to endocrine function need further investigation.

Case Reports

MARROW SCLEROSIS ASSOCIATED WITH MASSIVE MYELOID SPLENOMEGALY

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Marrow sclerosis appears to be a disease entity characterized by the following pathologic picture: fibrosis or bony sclerosis of the bone marrow, associated with extramedullary hemopoiesis in which megakaryocytes are numerous, leading to marked splenomegaly and some degree of hepatomegaly. In some cases there may be also gelatinous degeneration of the fatty marrow (Tudhope [1]).

The clinical picture is featured by leukoerythroblastic anemia, as Vaughan [2] termed it, or myelophthisic anemia, to use the term of the American writers. This anemia is of varying severity, with the presence of large numbers of nucleated red cells of the normoblast type and a small number of immature white cells of the granular series; the white cell picture varies from leukopenia to well marked leukocytosis but usually is within normal limits. Frequently present also are a hemorrhagic diathesis, an abdominal tumor (enlarged spleen), dyspnea, and edema of the legs, while a roentgenogram of the bones when properly controlled will often show some degree of sclerosis of the marrow, especially when the sclerosis is of a bony nature.

Such a clinical picture, except for the roentgen appearance, which unfortunately has seldom been ascertained in these cases, is very similar to that of the so-called aleukemic leukemia, particularly in a qualitative way, while the gross pathologic picture, except for the marrow changes, frequently simulates the latter condition, so much so that Hynes [3] recently suggested that myelosclerosis (marrow sclerosis) is probably the correct diagnosis in many cases previously reported as instances of aleukemic leukemia.

It is well known that leukoerythroblastic anemia can occur in a variety of conditions. For instance, Vaughan [2] found it in carcinomatosis of bone, Albers-Schönberg's disease (marble bones), multiple myeloma and Cooley's anemia, while Magner [4] wrote of it as being due to any "widespread invasion of red marrow by tumour, proliferating leukocytes or by fibrosis or bony tissue."

Because of these facts it is felt that more care should be taken in the pathologic and clinical study of the medullary cavity in any case presenting the main features outlined in the foregoing paragraphs, and for this reason a case of marrow sclerosis is reported.

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1. Tudhope, G. R.: J. Path. & Bact. **44**:99, 1937.
2. Vaughan, J. M.: J. Path. & Bact. **42**:541, 1936.
3. Hynes, M.: Quart. J. Med. **9**:177, 1940.
4. Magner, W.: Textbook of Hematology, Philadelphia, P. Blakiston's Son & Co., 1936, p. 166.

REPORT OF A CASE

A man aged 66 years was admitted to the Glasgow Royal Infirmary on Feb. 13, 1940, complaining of an ulcer on his right cheek of one year's duration, which had commenced as a small nodule, gradually increased in size, ulcerated and spread over the right malar area. On questioning it was elicited that he had a sense of fulness and flatulence with some epigastric discomfort following meals and was constipated and that he had noted a gradual loss of strength, with dyspnea on exertion, for about two years. There was no history of vomiting, hematemesis or pain.

An irregular septic sloughing ulcer, 4 cm. in diameter, overlay the right malar eminence. The edges were indurated. Later a biopsy proved it to be typical squamous carcinoma of Broders' grade 1, associated with suppuration and chronic inflammatory change. Both hands showed a few ecchymoses, which the patient stated usually appeared in cold weather and were unrelated to trauma. Nothing abnormal was noted in the cardiovascular, respiratory or genitourinary system. The abdomen was distended, and the border of the liver was found 2 inches (5 cm.) below the costal margin. The spleen was generally enlarged, the notch being both visible and palpable at the level of the umbilicus, but it was only slightly tender. No enlarged glands were found on careful examination.

The blood showed red cells 3,450,000, hemoglobin 55 per cent and white cells 5,800. The color index was 0.8. Stained films revealed marked hypochromic microcytic anemia; no nucleated red cells or immature white cells were detected. Urinalysis disclosed no sugar or albumin; no red cells, pus cells or casts were found on microscopic examination. A clinical diagnosis was made of squamous carcinoma of the cheek and splenic anemia.

In view of the postmortem observations it is unfortunate that no further hematologic study was made. This was due to the cutting down of all but essential investigations because of the smallness of the staff necessitated by the exigencies of war work.

Under appropriate treatment the sepsis involving the ulcer lessened greatly. The patient was up and around the ward and appeared quite comfortable. He had several minor attacks of epistaxis. Two days before his death he had a sudden hematemesis; a blood transfusion was given, but on the following morning a further small gastric hemorrhage occurred, followed almost immediately by a massive one, during which he died, Aug. 5, 1940.

The autopsy was held twelve hours after death. The body was that of an emaciated white man. There was marked pallor of the skin. A ragged ulcer was present over the right malar eminence, and several small ecchymotic patches were present in the skin of the hands.

The pleural sacs were normal; the lungs showed only marked edema. The heart weighed 290 Gm.; the valves and cavities appeared normal, but the myocardium was soft and flabby. There was no sclerosis of the coronary arteries or aorta.

The peritoneal cavity contained 500 cc. of clear amber ascitic fluid. The stomach contained a large quantity of bright red blood mixed with partially digested food; its mucosa was deeply blood stained, and several dilated veins were found in the cardiac end and in the lower part of the esophagus. No bleeding point was demonstrable, however, in spite of a careful search, the appearances being more suggestive of diffuse gastrostaxis. The duodenum, small intestine and cecum contained blood, which became darker in the lower portions of the tract; no ulceration or tumor was demonstrated in any region.

A firm mass of tissue was found in the retroperitoneal tissue posterior to the cecum, to which it was firmly attached; it measured 5 cm. in length and was adherent to the lower pole of the right kidney; on section it had a gray translucent

appearance and cartilaginous consistency. No enlarged glands were detected in the area, and there was no infiltration of the surrounding tissues. A similar but smaller piece of tissue was present at the lower pole of the left kidney.

The liver weighed 1,900 Gm. It was very firm, with a finely granular greenish yellow surface. The cut surface suggested some fibrosis, but there was no particular distribution. The gallbladder was normal, and the bile ducts were patent.

The spleen was greatly enlarged, weighing 2,050 Gm. and measuring 30 by 15 by 7.5 cm. The cut surface was reddish purple, and diffuse fibrosis was noted. No siderotic nodules were visible, nor were the appearances suggestive of lymphadenoma or myelogenous leukemia. The wall of the splenic vein was normal in size, and no obstruction was found.

The kidneys weighed 160 Gm. each. Scattered over the surfaces of both were several irregular hemorrhagic areas. These varied in size from some 3 mm. in diameter to larger, confluent patches measuring 3 by 2 cm. On section they were found to be in the capsule and could be stripped from the surface, there being no apparent infiltration of the kidney tissue. The larger areas varied from 1 to 4 mm. in thickness. The cortex and medulla were pale but otherwise appeared normal. Both pelves contained a few minute petechial hemorrhages.

The adrenals, pancreas, thyroid and testicles—all appeared normal. No abnormality was noted in the brain, which weighed 1,400 Gm.

The entire medullary cavity of the right femur was filled with fatty marrow; no hyperplastic foci were seen nor any thickening of the cortex or substantia spongiosa nor any fibrosis of the marrow. There was a very definite increase in the number and thickness of the bony trabeculae of the vertebrae, but no tumor nodules were detected. The medullary cavities of the ribs and sternum were filled with red marrow, but in addition bony sclerosis was present.

Histologic Examination.—Paraffin sections taken from many blocks were studied with hematoxylin-eosin Leishman, Dominici, Unna-Pappenheim and Masson [5] trichrome stains.

Spleen: The normal architecture was practically lost, only a portion of an occasional malpighian follicle remaining. There was slight diffuse fibrosis of the pulp, and the sinuses were packed with cells, a considerable number being of the giant cell type, the picture at first sight suggesting lymphadenoma. Study of the cells with the high power and the oil immersion lens, however, showed the majority of them to be normoblasts with an occasional megaloblast among them. In addition, many immature white cells were present, both myelocytes and myeloblasts being represented; the eosinophilic type of myelocyte predominated. Mature erythrocytes and polymorphonuclears were numerous. In each high power field were several spheroidal cells, larger than a megaloblast, with basophilic cytoplasm and large nuclei containing a reticular chromatin network; they appeared to be of the type described by Smith and Silberberg [6] as hemocytoblasts. The giant cells mentioned had abundant eosinophilic cytoplasm; in some there were from 2 to 6 nuclei clumped in the center and apparently joined to one another by thin bands, thus forming an irregular nuclear mass. These were taken to be megakaryocytes (fig. *A*).

The histologic picture was thus one of active hemopoiesis associated with early fibrosis and the presence of fairly numerous megakaryocytes.

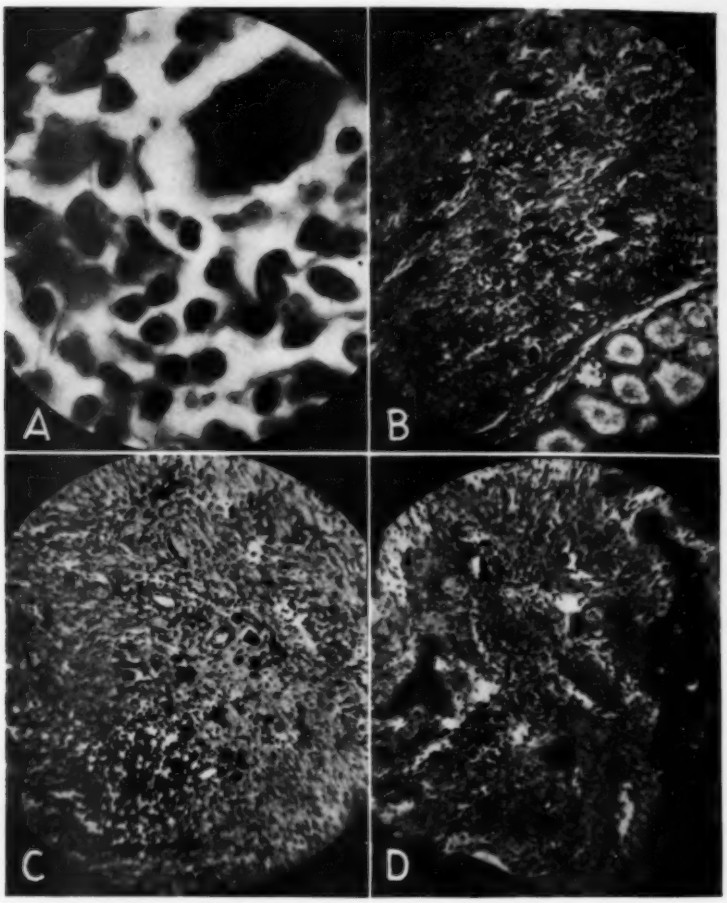
Liver: The sections revealed pronounced foci of necrosis, and in some of these areas young fibrous tissue formation was demonstrated; in several places strands

5. Masson, T.: J. Tech. Methods **12**:75, 1929.

6. Smith, R. P., and Silberberg, M.: Arch. Path. **21**:578, 1936.

of this tissue extended to the surface, at which point the capsule was dimpled, thus explaining the finely granular surface noted in the gross specimen. The sinusoids even in the necrotic areas contained many normoblasts, a few myelocytes and some megakaryocytes.

Kidneys: Sections through the hemorrhagic areas showed the capsule to be markedly thickened because of the proliferation of fibrous tissue containing many



A, photomicrograph of spleen under the oil immersion lens, showing hemopoiesis. Note the multinucleated megakaryocyte and numerous normoblasts.

B, photomicrograph of an area of the surface of a kidney under medium magnification, showing a portion of the kidney tubules and a portion of greatly fibrosed capsule, with hemopoietic cells and many megakaryocytes.

C, photomicrograph of an area of the tumor mass behind the cecum, under medium magnification, revealing fibrosis, a focus of lymphocytes and numerous megakaryocytes and small cells, which under higher power proved to be normoblasts and myelocytes.

D, photomicrograph of vertebral bone marrow, showing fibrosis and newly formed bony trabeculae.

capillaries. Scattered throughout this tissue were numerous normoblasts, immature white cells, megakaryocytes and hemocytoblasts (fig. *B*). For the most part they were diffusely arranged but in places occurred in small foci. In one area the superficial cortex showed early infiltration by these cells.

Tumor Mass at Lower Poles of Kidneys: This was found to consist of a collagenous fibrous tissue in which were numerous foci of cells similar in all respects to those found in the capsules of the kidneys (fig. *C*); they had for the most part a perivascular distribution in relation to young capillaries. Here and there were some small areas of lymphocytes suggesting that this focus of active hemopoietic tissue had probably arisen from the vestigial remains of the hemolymph glands.

Bone Marrow: Sections from several blocks taken from the sternum and vertebrae showed the same changes. There was a definite increase of bony trabeculae, and between them was a proliferation of fibroblasts laying down collagen. Scattered in this fibrous tissue were typical young marrow cells (fig. *D*). There was, however, no hyperplasia of the marrow elements, and megakaryocytes were not so numerous as in the other organs described.

The marrow of a femur was completely fatty with only an occasional focus of inactive hemopoietic cells. These apparently were even less numerous than in the normal quiescent marrow of the adult. The marrow revealed no evidence of either bony or fibrous sclerosis.

COMMENT

The essential pathologic change in this case consisted of sclerosis of the marrow of the vertebral bodies and sternum and complete inactivity of the marrow of the long bones, with active extramedullary hemopoiesis in the spleen, liver, renal capsules and vestigial remains of the hemolymph glands in the retroperitoneal tissues. This was associated with massive splenomegaly and hepatomegaly, the liver showing, in addition, focal necrosis and early fibrosis.

The case thus appears to agree with the diagnosis of marrow sclerosis except that the blood picture was not of the leukoerythroblastic type; it has already been pointed out, however, that only one count was carried out, and McMichael and McNee [7] stated that in this condition the hematologic features undergo such extreme variation and fluctuation that the clinical diagnosis is rendered difficult unless frequent blood counts are done. In addition, in this case the marrow of the long bones was inactive and showed no sclerosis.

Other cases which appear to be similar from both clinical and pathologic standpoints have been reported under a great variety of names, e. g.: "splenomegaly with myeloid transformation" (Tudhope [1]); "leuko-erythroblastosis" (McMichael and McNee [7]); "megakaryocytic myelosis with osteosclerosis" (Hewer [8]); "myelophthisic splenomegaly" (Ballin and Morse [9]; the roentgenograms of marrow cavities appeared normal in their cases, but no histologic examination was done); "splenomegaly with sclerosis of bone marrow" (Donhauser [10]); "fibrosis of bone marrow" (Mettier and Rusk [11]); "osteosclerotic anemia" (Chap-

7. McMichael, J., and McNee, J. W.: Edinburgh M. J. **43**:303, 1936.
8. Hewer, T. F.: J. Path. & Bact. **45**:383, 1937.
9. Ballin, M., and Morse, P. F.: J. A. M. A. **89**:1671, 1927.
10. Donhauser, J. L.: J. Exper. Med. **10**:559, 1908.
11. Mettier, S. R., and Rusk, G. Y.: Am. J. Path. **13**:377, 1937.

man [12]); "splenomegalie myeloide megakaryocytaire amelocythemia" (Weil, Chevallier and Sée [13]); "chronic non-leukemic myelosis" (Hickling [14]). This wide diversity of nomenclature appears to indicate that the essential pathologic change in marrow sclerosis has not been fully appreciated and again emphasizes the importance of more careful study of the bone marrow in all such cases.

NATURE OF THE DISEASE

Various etiologic factors have been suggested as causes of marrow sclerosis, and some of the more logical ones will be commented on here.

Donhauser,[10] who was one of the first to report a case, suggested that some unknown toxin acts on the marrow, causing the fibrosis; i. e., there is a chronic inflammatory change as the result of which there is a reversion to blood formation in the fetal blood-forming foci. This theory seems to be the most favored. Firket and Campos [15] described an interesting case, similar in all respects to those cited earlier in this paper, except that the marrow of the long bones was aplastic instead of sclerotic. Their patient had been exposed to fumes of benzene for a year, four years before admission to the hospital. In studying the cause experimentally they produced myeloid metaplasia in the spleen, liver and other organs with a marked megakaryocytic reaction by the injection of saponin in rabbits, thus supporting the theory that the disease is due to the action of a toxin.

Mettier and Rusk [11] speculated that since in several reported cases there was a leukemoid picture (in one of their cases the disease was termed myelogenous leukemia; in the other, aleukemic leukemia), the fibrosis of the marrow may result from the irritation of the hyperplastic marrow cells. The irritative effect is also favored by Hynes [3] and Hewer.[8] This theory is borne out in a case of polycythemia vera recorded by Hirsch; [16] this case was observed over a period of thirty-one years, and finally marrow sclerosis developed, with the usual pathologic picture. Vaughan and Harrison [17] reported a similar case.

If this conception were accepted, i. e., that there is first great hyperplasia of the marrow cells, followed by fibrosis, one would expect that typical leukemia would terminate in this way in more cases or that marrow sclerosis would more frequently present a typical leukemoid blood picture. These do not hold true, even though Goodall [18] cited the case of a 10 week old infant with myelogenous leukemia associated with marrow sclerosis. Thus, even though medullary sclerosis may occur occasionally in this fashion it is felt that when it does it should be excluded from the group defined under the entity of marrow sclerosis as outlined at the beginning of this article.

Of Hickling's [14] cases of chronic nonleukemic myelosis, 2 showed definite bony changes, while in the others roentgenograms revealed no "notable abnormality." However, in none of the cases was a thorough

12. Chapman, E. M.: Am. J. M. Sc. **185**:171, 1933.
13. Weil, P. E.; Chevallier, P., and Sée, G.: Sang **7**:773, 1933.
14. Hickling, R. A.: Quart. J. Med. **6**:253, 1937.
15. Firket, J., and Campos, E. S.: Bull. Johns Hopkins Hosp. **33**:271, 1922.
16. Hirsch, E. F.: Arch. Path. **19**:91, 1935.
17. Vaughan, J. M., and Harrison, C. V.: J. Path. & Bact. **48**:339, 1939.
18. Goodall, A.: Edinburgh M. J. **8**:500, 1912.

histologic examination of the marrow cavities made. In one of the two autopsies performed only the vertebral bodies were examined, while in the other only the lower end of the femur was studied. Although he emphasized the massive myeloid splenomegaly and metaplasia in the other organs as being the essential change, one feels that marrow sclerosis cannot be ruled out on the basis of such an incomplete examination of the bones. In discussing the causes he pointed out that blood uric acid and bilirubin are frequently high, indicative of increased destruction of blood. His suggestion is that this excessive destruction of blood might be a compensatory process in a case which otherwise might present a leukemic picture, or that it might be the primary process and the myeloid metaplasia in the organs an attempt to compensate for it. This is comparable to the extramedullary hemopoiesis sometimes found in pernicious anemia, mentioned by Lyall.[19]

Two points are of interest in this theory of increased blood destruction; first, Vaughan [17] found an increase in red cell fragility associated with an increase in cell thickness in 2 cases of marrow sclerosis studied by her. She offered no comment on this until further cases are examined. Second, several cases similar to those under discussion have shown a definite and often marked rise in the white cell count following splenectomy, the picture becoming leukemic in character; e. g., Hickling [14] cited 5 such cases. Pinkerton [20] reported a case in which the count rose from 5,200 to 180,000 per cubic millimeter, with 83 per cent myelocytes; in a case studied by Ballin and Morse [9] there was an increase to 52,000 per cubic millimeter; no myelocytes were present but normoblasts were numerous. Finally Mettier and Rusk [11] reported a case in which the count rose from 4,200 to 25,900, with 54 per cent myelocytes. The latter cases suggest that the reticuloendothelial system as present in the spleen may be destroying an excess of blood cells or at least holding them in check in some way and preventing their appearance in the blood stream. However, it is possible that the blood cells are merely stored in the splenic sinusoids, since it has been shown by Weil and co-workers [13] and Chapman [12] that in their cases of marrow sclerosis the injection of a solution of epinephrine hydrochloride caused a momentary increase in the white cell count, with the appearance of more numerous immature cells in the blood stream.

The last suggestion is probably the correct view, for one cannot conceive of the reticuloendothelial system destroying the excess of white cells as suggested in the foregoing paragraph and the spleen at the same time reverting to its blood-forming capacity and forming more cells. The last suggestion would also explain the fluctuation of the white cell count, which so frequently occurs.

Still another theory has been suggested by Wolf,[21] namely, that marrow sclerosis may be the end stage of a disease that begins with atrophy of the marrow. This would be in keeping with the finding in several cases that the marrow of the long bones was still fatty instead of being hyperplastic and sclerotic, e. g., in the case reported by McMichael and McNee [7] and the one presented here. It should be noted, however, that Turnbull [22] showed that the sclerosis need not be present throughout all the bones in order to give the typical picture.

19. Lyall, A.: J. Path. & Bact. **41**:469, 1935.
20. Pinkerton, H.: Arch. Path. **7**:567, 1929.

(Footnote continued on next page)

Finally, Vaughan [17] expressed the belief that some as yet unkown stimulus leads to both the leukoerythroblastic anemia and the marrow sclerosis. She pointed out that all the cells involved—osteoblasts, fibroblasts, hemocytoblasts and megakaryocytes—arise from the primitive mesenchymal cells of Maximow, the proliferation of the former cells giving the sclerosing effect while the latter cells give the extramedullary hemopoiesis and the special type of blood picture. This view, at least as far as the megakaryocyte is concerned, is supported by Downey, Palmer and Powell,[23] who in reporting a similar case of leukoerythroblastic anemia concluded that the megakaryocytes present in the metaplastic areas arose in situ from "the reticulum cells and myeloblasts" rather than as embolic deposits from the marrow.

After careful consideration and review of all these theories and of the present case it is felt that marrow sclerosis can be classified only as belonging to the still far from understood dyscrasias of the reticuloendothelial system. The focal necrosis in the liver in the case presented rather supports the view that the underlying agent involved is of toxic nature, here possibly bacterial but also possibly chemical. Whatever the causative agent is, it has a depressing action on the normal blood-forming elements of the bone marrow, in some cases merely causing inactivity, as seen in the long bones of the present case, and in others giving rise to a proliferation of fibroblasts and osteoblasts (in the sternum and vertebrae as described in this paper). In consequence there is a reversion to the fetal hemopoietic function, with hyperplasia of the mesenchymal elements, resulting in the extramedullary marrow formation in the spleen, liver and possibly hemolymph glands. This may or may not manifest itself in the circulating blood.

As an aid to diagnosis it is emphasized that in all cases presenting the main clinical features of marrow sclerosis one should do frequent blood counts, that the marrow cavities should be thoroughly examined by means of roentgenograms and marrow punctures and that, finally, the injection of a solution of epinephrine hydrochloride to force cells out of the spleen into the blood stream may be of help when the blood picture is not of the typical leukoerythroblastic type. In all autopsies of persons dying of obscure blood dyscrasias a thorough histologic examination of the medullary cavities of the long bones, vertebrae, sternum and ribs should be routinely carried out.

SUMMARY

A case of marrow sclerosis in which there was extramedullary hemopoiesis in the spleen, renal capsules, liver and possibly in the hemolymph glands, with fibrosis and osteosclerosis of the marrow of the sternum and vertebrae, is presented.

The necessity for frequent blood counts and a complete investigation of all the bones in obscure blood dyscrasias is emphasized.

The possible causation and nature of marrow sclerosis are considered.

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21. Wolf, C.: Beitr. z. path. Anat. u. z. allg. Path. **89**:151, 1932.

22. Turnbull, H. M.: Pathologic Report, in Hunter, D.: Proc. Roy. Soc. Med. **28**:1629, 1935.

23. Downey, H.; Palmer, M., and Powell, L.: Folia haemat. **41**:55, 1930.

HEMANGIOENDOTHELIOMA OF TIBIA WITH METASTASIS TO THE POPLITEAL ARTERY

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Great confusion has arisen with regard to tumors of blood vessels in long bones on account of the cases reported as instances of primary blood vessel tumors despite the failure to demonstrate a derivation from blood vessels and despite the definite epithelial character of some of the tumors. Ribbert [1] stated that most of the cases of so-called endothelioma of bone were instances of epithelial tumor. Other examples of the epithelial-like tumor of bone are offered in the cases of Renton and Teacher,[2] Kolodny [3] and Lutz and Pusch.[4] In discussing the case reported by the last-named authors Geschickter [5] stated that the tumor resembled a metastasis from hypernephroma of the kidney but cited a case of this type of bone tumor in which no other tumor was found at autopsy. He brought forward the interesting suggestion that tumors of this type arise from the primitive mesoderm about the joints, which is related to the mesothelium of the pleura and to the synovial membrane. If this suggestion is valid, these tumors should be separated from those arising from blood vessels, and the terms "hemangioma," "hemangioendothelioma" and "angioendothelioma" should not be applied to them.

Authentic hemangioma of a long bone has been infrequently reported in the literature. Bucy and Capp [6] in 1930 were able to collect only 7 cases of this type of tumor. Of the 4 cases reported by Geschickter and Maseritz [7] in 1938, only 2 are undoubtedly instances of hemangioma. Since publication of the latter report, 2 doubtful cases have been recorded by Dussaut [8] and 1 case, which has been accepted by the Registry of Bone Sarcoma, by Hauser.[9]

Most of the reported authentic cases have been instances of benign mature capillary or cavernous hemangioma. Included among these are the cases of Bucy and Capp,[6] Hitzrot,[10] Ballance and Shattock,[11] Pohl,[12]

From the Laboratory of Pathology and Department of Surgery, Cancer Section, Westfield State Sanatorium, Massachusetts Department of Public Health.

1. Ribbert, H.: Geschwulstlehre, Bonn, Germany, F. Cohen, 1914, p. 245.
2. Renton, J. C., and Teacher, J. H.: Glasgow M. J. **63**:368, 1905.
3. Kolodny, A.: Arch. Surg. **12**:854, 1926.
4. Lutz, J. F., and Pusch, L. C.: J. A. M. A. **113**:1009, 1939.
5. Geschickter, C. F., in discussion on Lutz and Pusch.[4]
6. Bucy, P. C., and Capp, C. S.: Am. J. Roentgenol. **23**:1, 1930.
7. Geschickter, C. F., and Maseritz, L. H.: J. Bone & Joint Surg. **20**:888, 1938.
8. Dussaut, A.: Día méd. **11**:146, 1939.
9. Hauser, H.: Am. J. Roentgenol. **42**:656, 1939.
10. Hitzrot, J. M.: Ann. Surg. **65**:476, 1917.
11. Ballance, H., and Shattock, S. G.: Brit. J. Surg. **11**:622, 1924.
12. Pohl, R.: Fortschr. a. d. Geb. d. Röntgenstrahlen **39**:1099, 1929.

Maugière [13] and the 2 authentic cases of hemangioma of Geschickter and Maseritz.[7] The malignant, anaplastic hemangioma or hemangioendothelioma has been conspicuous by its extreme rarity. Hauser's [9] case falls into the latter category.

This preponderance of benign hemangioma has created doubt on the part of many authors as to the existence of malignant blood vessel tumors of bone. Codman [14] in 1925 stated that many of the supposed cases of angiosarcoma were probably instances of telangiectatic osteogenic sarcoma. Bucy [15] and Bucy and Capp [6] in a review of the literature could find no record of either metastasis or recurrence of a tumor of this type and stated that no mitosis was ever seen in such a tumor. They expressed a doubt whether a malignant hemangioma of bone or a malignant transformation of a benign hemangioma of bone had ever occurred.

This impression that all tumors of long bones diagnosed as hemangioma are benign has led to the unfortunate recommendation that all such tumors be treated as benign growths. For example, Bucy and Capp [6] and Cutler and Buschke [16] have insisted that amputation is never indicated.

On account of the rarity of the malignant type of blood vessel tumor, or hemangioendothelioma, of a long bone and the scarcity of knowledge concerning the proper treatment, the following case of hemangioendothelioma of a tibia metastasizing to the popliteal artery is offered for consideration.

REPORT OF A CASE

A 50 year old white man entered the Westfield State Sanatorium on July 13, 1938 for high voltage roentgen irradiation of a lesion of the left knee. About eight months previously he had noticed a "tired" sensation in the left knee, and later he began to limp. About a month after this, a diffuse swelling was noted around and just above the left knee. The lesion noted in a roentgenogram taken outside this hospital was diagnosed by a competent roentgenologist as either a giant cell tumor or a myeloma, with probably no malignant tumor present. A biopsy made outside led to the conclusion that no tumor of any importance was present.

On admission of the patient to the Westfield State Sanatorium, a hard warm diffuse swelling was observed over the upper end of the left tibia, with a well healed scar, 6 cm. in length, over the midportion of the swelling. A roentgenogram revealed multiple trabeculations in the proximal portion of the tibia. The medial two thirds of the proximal tibial cartilages and both the anterior and the posterior tibial spines were involved by the neoplasm. A break in the continuity of the medial surfaces of the cortex was thought to be due to the previous operation. A minute punched-out round shadow was found in the subtrochanteric region of the right femur, and this was interpreted as developmental in character. A diagnosis of benign giant cell tumor was made. Roentgenograms of the chest and intravenous pyelograms revealed nothing of importance.

The urine was normal. The white blood cell count was 7,800, with neutrophils 73 per cent, lymphocytes 24 per cent and eosinophils 3 per cent; the red blood

13. Maugière, L.: J. de radiol. et d'électrol. **4**:269, 1920.

14. Codman, E. A.: Bone Sarcoma, New York, Paul B. Hoeber, 1925.

15. Bucy, P.: Am. J. Path. **5**:381, 1929.

16. Cutler, M., and Buschke, F.: Cancer: Its Diagnosis and Treatment, Philadelphia, W. B. Saunders Company, 1938, p. 641.

cell count was 4,870,000 and the hemoglobin content 90 per cent. The red cells and platelets were normal. The blood serum protein was 7.8 Gm. per hundred cubic centimeters, and the albumin-globulin ratio was 2:1. The blood serum calcium was 10.8 mg. per hundred cubic centimeters.

A curettement was done July 29. The definite anaplasia of the tumor made it doubtful if high voltage roentgen rays would be effective. Amputation was advised. A left mid-thigh amputation was done by one of us (F. H. B.) August 22. The patient when last seen, May 29, 1940, showed no evidence of recurrence or metastasis clinically or by roentgenogram.

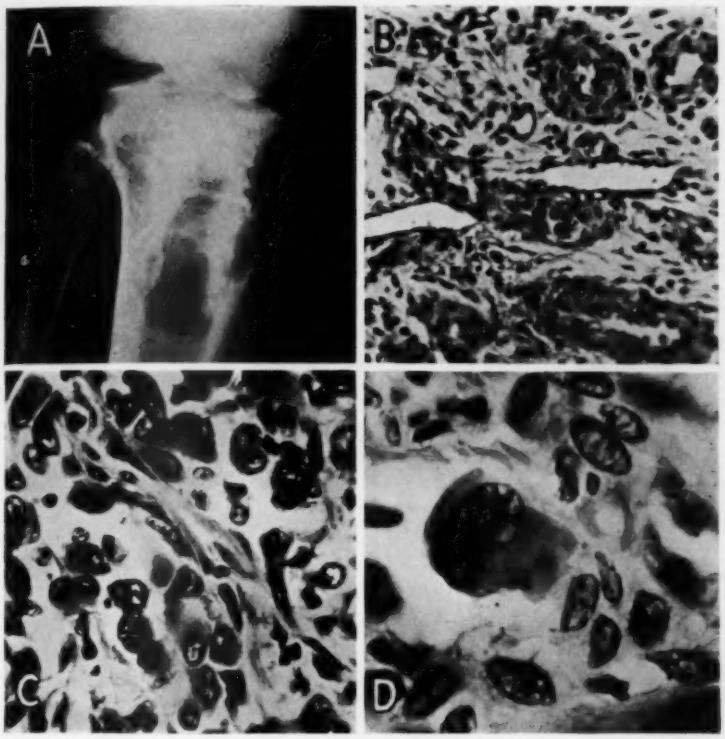

Fig. 1.—*A*, roentgen appearance of the upper portion of the left tibia, which is involved by hemangioendothelioma. Note the large loculations. *B*, well differentiated portion of the hemangioendothelioma of the tibia; × 200. Note the blood vessels with thick and thin walls. *C*, poorly differentiated portion of the hemangioendothelioma of the tibia, with proliferation of endothelial cells within the blood vessels; × 400. *D*, large intimal neoplastic giant cell with multilobated nucleus found in an anaplastic region of the hemangioendothelioma of the tibia; × 900.

The curettings obtained July 29, 1938 consisted of several pieces of reddish purple, clotlike, soft to firm glistening tissue with occasional small, ill defined yellowish white patches of fibrosis together with small fragments of cancellous and cortical bone. The entire mass measured about 9 cm. in diameter.

The lower portion of the femur was amputated 18 cm. above the external condyle. The amputation site was without unusual features. On the dorsum of the fourth toe and the inner surface of the left foot were found areas of dark brown pigmentation, measuring 0.2 to 4 cm. in diameter. On the medial surface of the tibia just below the patella was an operative incision, 6 cm. in length, leading to a cavity 7 cm. in diameter. Extending downward from the open operative incision for a distance of 4 cm. was a well healed scar. The cavity was partly lined by dark, reddish purple, clotlike tissue, similar to the curetted tissue described, with invasion of the cortical bone. The cortex forming the wall of the cavity showed in its posterior and posteromedial portions defects measuring up to 3 cm. in diameter. These defects were filled with fibrous tissue containing masses of the clotlike tissue. Clotlike tissue invaded the epiphysis and the anterior and superior tibial spines. The articulating surfaces of the knee joint were smooth, but there were softening and dull golden brown discoloration of the cartilage about the attachment of the anterior cruciate ligament, where the infiltrating clotlike tissue had almost reached the surface. In the popliteal space were two lymph nodes, about 1 cm. in diameter, consisting partly of firm brown glistening tissue and partly of yellow fatty tissue. The popliteal artery and the adjacent portion of a small branch at the level of the tibial condyles were thickened, and the perivascular tissue showed brownish discoloration. A direct extension from the tumor in the tibia to the thickened portion of the popliteal artery was not noted grossly.

MICROSCOPIC APPEARANCE OF TUMOR

The tissues were stained with hematoxylin and phloxine (tetrabromdichlorfluorescein), and with phloxine and methylene blue. The neoplastic tissue in the curettings and in the cavity of the amputated tibia presented the same histologic details. Both comparatively acellular fibrous regions and hemorrhagic anaplastic cellular regions were present. The cellular regions predominated.

The fibrous regions were both edematous and dense. Large foci of necrosis were occasionally seen. In addition, these fibrous regions contained well formed, widely spaced, rather small blood vessels of arterial, arteriolar and venous types, as well as infrequent small groups of well formed capillaries. The vessels were filled with red cells. The arterial and arteriolar vessels possessed thick walls, with peripheral condensed collars of collagen fibers. The walls within these collagen collars consisted of one to several layers of swollen intimal endothelial cells. Often, the swollen endothelial cells in the innermost layer protruded into the lumen of the vessel, almost obliterating it. The endothelial nuclei varied moderately in shape, with oval and indented vesicular nuclei visible. Occasional small nucleoli were present. The cytoplasm was acidophilic, finely granular and at times abundant. Desquamation of the endothelial cells into the lumen was noted. The venous type of vessel had thin walls lined by elongated flat cells arranged in a single layer. The cytoplasm was scanty. The nuclei were spindle shaped and vesicular. At the periphery of the fibrous regions the well developed vessels merged with the cellular anaplastic portions. Visible in the fibrous stroma were scattered plasma cells, lymphocytes and phagocytes laden with hemosiderin granules.

The cellular regions showed not only small, well formed capillaries but, in addition, anaplastic areas where the capillary structure became

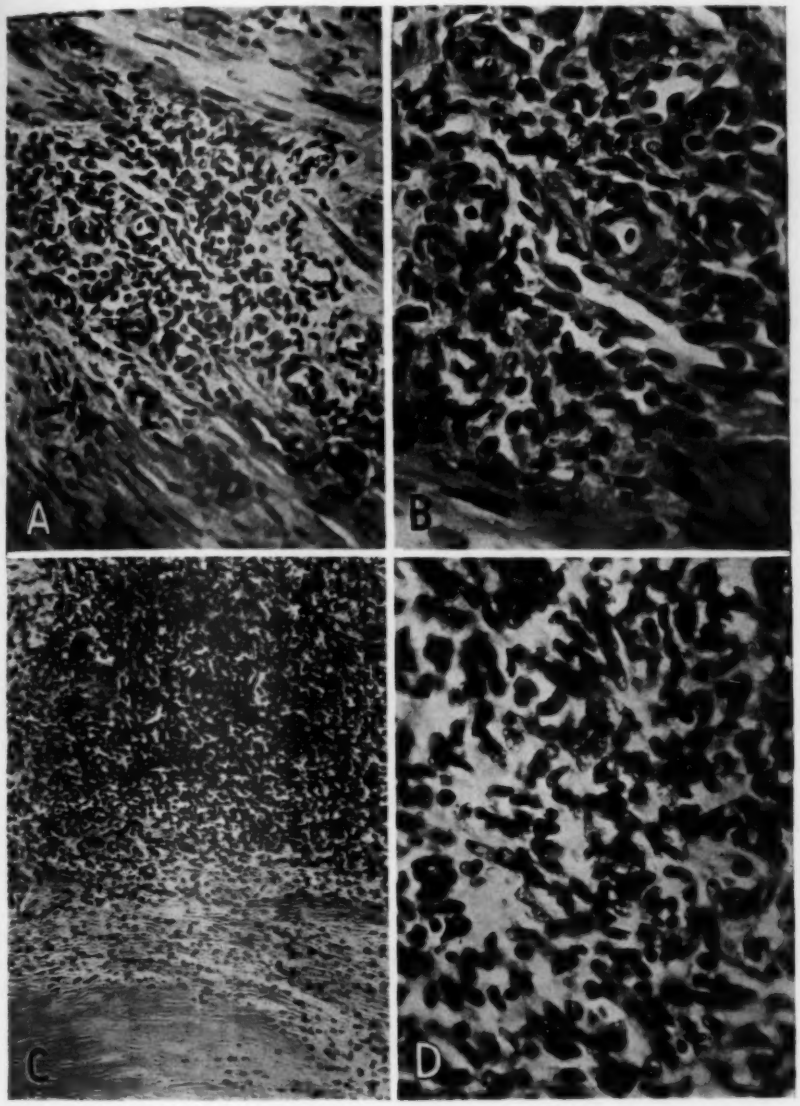

Fig. 2.—*A*, section passing through the media of the involved popliteal artery; × 200. Note the metastasis of the hemangioendothelioma of the tibia invading the media. *B*, field from the metastasis in *A* at higher magnification, showing anaplasia with blood vessel formation still present; × 400. Smooth muscle cells of the media may be seen below. *C*, section passing through the intima and media of the popliteal artery; × 100. Note the metastasis of the hemangioendothelioma of the tibia invading the media and encroaching on the intima below. *D*, field from the metastasis in *C* at higher magnification, showing marked anaplasia, with only a rare capillary containing red cells present; × 400. Note the irregularity of the arrangement of the neoplastic cells.

less well defined. Transitional areas, where the well formed capillaries merged with the anaplastic cell masses, were abundant.

The well formed small capillaries in these regions were filled with red cells and were often separated by extravasated red cells. Narrow fibrils could often be seen about the capillaries. In the anaplastic area, the endothelial cells proliferated within the capillary vessels, obliterating the lumens; often they formed both compact and loosely arranged syncytial masses with well separated small clefts filled with red cells, representing capillary spaces. Numerous extravasated red cells were present in such areas.

In these cellular regions there was marked pleomorphism of the endothelial cells. Large cells with large hyperchromatic multilobated nuclei were found scattered among the more numerous cells with small oval and spindle-shaped vesicular nuclei containing fine scattered chromatin granules. The giant nuclei contained multiple prominent nucleoli. Some curious cells resembling the giant cell seen in a foreign body giant cell tumor were found infrequently within the lumen of a dilated capillary, apparently proliferating from the intimal cells. These multinucleated cells were found far from any focus of foreign body giant cell reaction. The outlines of all the cells were irregular and rather indistinct, with wide elongated forms often seen. The cytoplasm of the cells was frequently abundant, acidophilic, finely granular and at times vacuolated. Nowhere were there epithelial-like cells with clear cytoplasm and rigid cell membranes. Mitotic figures were moderately frequent, about one to a low power field in some regions.

Sections through the intact cortex at the site of the tumor showed neoplastic tissue infiltrating the marrow between the thickened bony trabeculae. In other regions the cortex had been fragmented and the neoplastic tissue had extended through the periosteum to involve the adjacent tendinous tissues. A few bony spicules within the main mass of neoplastic tissue were undergoing osteoblastic and osteoclastic activity, with the former more pronounced.

In the thickened fibrotic periosteum an occasional focus of foreign body giant cell reaction was found, with fragments of suture material present.

A section through the thickened portion of the popliteal artery was seen to run also through the proximal portion of a branch. Within the popliteal artery, close to the mouth of the branch, a metastasis was found occupying the entire width of the media and encroaching on the intima and adventitia. The metastatic tissue was identical with that in the more anaplastic portions of the primary tumor in the tibia, with blood vessel formation still visible. Scattered masses of hemosiderin and eosinophils were noted within the neoplastic tissue. A similar but separate focus of neoplastic tissue was observed in the branch, but here only a single layer of intimal endothelial cells was present between the lumen and the metastatic tissue, which extended into the lumen in the shape of narrow folds. Despite careful examination, no evidence of direct extension from the primary tumor in the tibia to the neoplastic tissue in the popliteal artery and its branch could be found; in other words, this focus represented a true metastasis. The popliteal vein was not involved.

The lymph nodes found in the popliteal space contained no neoplastic tissue.

COMMENT

In view of the uniqueness of this tumor it is essential that the criteria on which the diagnosis is based be examined with great care. That the tumor was primary in bone is shown by the fact that evidence of a primary hemangioendothelioma elsewhere invading or metastasizing to the tibia could not be found, the main tumor mass being confined to the tibia. Microscopic sections revealed that the main feature of the tumor was the blood vessels, ranging from well to poorly differentiated types. Even in the most anaplastic regions evidence of blood vessel formation was found. As far as malignancy is concerned, all the criteria needed for such a diagnosis were present—anaplastic regions, pleomorphism of cells and mitotic figures. Final confirmation of the malignancy of the tumor was had in the metastasis to the popliteal artery. The destruction of the cortex and the invasion of the extraperiosteal tissues were also in agreement with the presence of a malignant tumor but might have been caused by the operative intervention.

As in most cases of hemangioma of a long bone, the site was at the end of the bone, in the epiphysial region. This site as well as the prominent loculations seen by roentgenogram made a differential diagnosis between hemangioma and benign giant cell tumor necessary. In this connection, as far as benign hemangioma of a long bone is concerned, Bucy and Capp [6] stated that loculations of hemangioma of a long bone are smaller than those of a benign giant cell tumor and within them a fine fibrillary framework is present. They also found that in the presence of a benign giant cell tumor the cortex is expanded, while in that of hemangioma it is partially eroded. Geschickter and Maseritz [7] also noted that hemangioma tends to progress and be more extensive than a benign giant cell tumor. In addition, they stated that the loculations in hemangioma are bordered by a heavier framework of osseous tissue than in a benign giant cell tumor. It should be noted that in roentgenograms hemangioma of a long bone sometimes simulates bone cyst.

With regard to the malignant types of hemangioma of a long bone, few data concerning the roentgen appearance are available. As far as the case under discussion is concerned, here, too, the roentgen appearance was not unlike that of a benign giant cell tumor, as the diagnosis by several competent roentgenologists demonstrated. On the other hand, unlike the benign hemangioma of a long bone in the cases cited in the foregoing paragraph, the neoplasm in this case had quite large loculations and invaded the tibial articular cartilages. Of course, in the final analysis an examination of the tissues under the microscope is absolutely necessary to establish the correct diagnosis.

It must be emphasized, however, that not only the diagnosis but also the future course of therapy is determined by the histologic findings. In addition, the site of the tumor in the bone must not be neglected. For instance, in the case reported here, the anaplasia present in the tumor made it seem probable that roentgen radiation would have slight effect. Dr. Shields Warren reviewed the slides and concurred in this opinion.[17] Resection, on the other hand, would have been impossible, owing to the anatomic site and the involvement of the articular cartilages

17. Warren, S.: Personal communication to the authors.

of the tibia. It was difficult to see how a useful, weight-bearing limb could be obtained by resection alone. For these reasons amputation was advised.

Subsequent examination of the amputated limb disclosed that the decision to amputate based on the histologic findings was a wise one. Indeed, the finding of the metastasis in the popliteal artery confirmed the belief in the futility of a trial of high voltage roentgen radiation, for the damage to the arterial wall following irradiation, supposing that the tumor were radiosensitive, might have resulted in an aneurysm or at least in fibrosis of the wall with obstruction to the blood flow. As for resection, this procedure would have left the metastasis in the popliteal artery, and a recurrence as well as other metastases along the course of the popliteal artery might well have occurred. There can be no question that the rule that amputation should never be done for hemangioma of a long bone must be abandoned in the light of the findings in our case. Certainly, instead of dogmatically insisting that all such tumors be treated alike, one should recommend a study of the histology of the tumor. Only after consultation with the pathologist should the surgeon proceed to carry out the treatment.

SUMMARY

A primary tumor of the tibia is reported as an authentic malignant anaplastic hemangioma or hemangioendothelioma which metastasized to the popliteal artery. The diagnosis of this rare type of tumor of bone can be made only by biopsy, since the roentgenologic appearance simulates that of a benign giant cell tumor. A careful consideration of the histologic details is necessary to separate the benign from the malignant blood vessel tumors of bone. It is suggested that not all tumors of the long bones diagnosed as hemangioma be regarded as benign and treated as such, despite the opinion prevalent in the literature. Amputation, which proved to be the best treatment in the case reported, is suggested as the method of choice for a malignant blood vessel tumor of a long bone.

CONGENITAL BACILLUS PYOCYANEUS INFECTION

E. J. Kraus, M.D., and Mary P. Hunter, M.D., Peoria, Ill.

Formerly, Bacillus pyocyaneus was considered an organism without much pathogenicity. But increasing numbers of reports have shown that it is particularly virulent in children and infants and to a less extent in adults. In the latter, it is frequently found as a cause of infection in genitourinary diseases [1] and has also been isolated in such conditions as acute bacterial endocarditis, ocular infections, enteritis, gangrenous skin lesions and septicemia.[2]

In poorly nourished children this bacillus is associated with infections of the ears, skin, respiratory, digestive and genitourinary tracts, meninges, bones and eyes.[3]

In undernourished and feeble infants a general infection may occur, which often terminates fatally. It may come from any primary focus, the commonest sources [4] being the intestinal tract, middle ear, nasal cavity and throat. Several cases of recognized B. pyocyaneus septicemia have occurred in infants with hereditary syphilis.[5]

In the newborn, the umbilical wound seems to be the commonest portal of entry, Wassermann having reported an epidemic of 11 cases with this history.[6] In an extensive survey of the literature there was found only 1 case of B. pyocyaneus infection of the newborn which was thought to be congenital. The mother was suffering from septic endometritis at delivery. On the fourth day signs of meningitis developed in the infant. After three weeks pronounced hydrocephalus had developed and B. pyocyaneus was isolated by ventricular puncture. Only the brain was examined at autopsy, on the thirty-seventh day.[7] In view of the apparent rarity of congenital B. pyocyaneus infection we present a case, in which we were able to trace the source of infection and study the bacteriologic and morphologic aspects.

From the Laboratory of Pathology and Bacteriology, St. Francis Hospital.

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2. Fraenkel, E.: Virchows Arch. f. path. Anat. **183**:465, 1906; Ztschr. f. Hyg. u. Infektionskr. **72**:486, 1912; **84**:369, 1917. Kearns, J. J.: Arch. Path. **21**:839, 1936. Thayer, W. S.: Edinburgh M. J. **38**:237, 1931. Thorsen, G.: Acta obst. et gynec. Scandinav. **18**:490, 1938.

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REPORT OF A CASE

A full term boy died twenty hours after birth. On the day prior to delivery of the baby, the mother (para II, gravida III) suddenly became drowsy and chilly, perspired profusely and had to go to bed for four hours. During the next twelve hours she had three bowel movements, with stools yellowish brown and slightly watery but containing no blood. These movements were accompanied by generalized colicky abdominal pains. For the next six hours she was in labor. During this time she had two severe chills, after each of which she felt feverish.

The baby weighed 7 pounds 12 ounces (3,515.5 Gm.). Over the whole body were areas of a macular type of rash. These areas were reddish purple and of various sizes and shapes, some being 6 cm. in diameter. The baby had difficulty in breathing and was restless and cyanotic. Shortly after birth there were 3,000 white cells per cubic millimeter of blood, 91 per cent of which were lymphocytes. In spite of the administration of oxygen and of 40 cc. of 2 per cent dextrose in Ringer's solution by hypodermoclysis, the baby died twenty hours after birth.

At postmortem examination, one hour after death, the body was that of a well developed newborn white boy 52 cm. in length. The face was strikingly pale; the skin on the trunk and limbs was purple mottled, without signs of the premortem rash. The abdomen, which was slightly distended, contained a small amount of sticky yellowish exudate, containing thin threads of fibrin. In each pleural cavity there was about half a teaspoonful of seropurulent fibrinous exudate; the pleura showed many petechiae. The pericardium also had several petechiae. The subarachnoid space at the base of the brain, especially in the sylvian fossae, contained diffuse hemorrhages. The brain was soft and pale. The kidneys were soft in consistency and showed a red-mottled marking.

Cultures of the blood, the spleen, the pleural exudate and the hemorrhagic exudate at the base of the brain gave pure growths of B. pyocyaneus.

Cultures from the feces of the mother directly after childbirth produced many colonies of B. pyocyaneus, with the same cultural characteristics as the strain isolated from the baby. Agglutination tests with the strain from the baby and the mother's serum were negative. Blood cultures and cultures from the lochia were negative. The positive cultures showed the characteristics of B. pyocyaneus, such as typical small green colonies, motility of the organism, hemolysis of blood agar and acid coagulation of litmus milk. Both strains produced a slight reduction of nitrates to nitrites. No indole was formed. The two pigments pyocyanine and fluorescein were extracted from the organisms.

Histologic Examination.—The lungs showed for the most part a widening of the pleura and interlobular septums. In places these were eight to ten times as wide as normal (fig. 1). The widening was due to marked serous exudation pushing the collagenous fibers far apart. Within this edema-like effusion were moderate numbers of mononuclear wandering cells, polymorphonuclears, lymphocytes and sparse erythrocytes, together with a small amount of fibrin. In some places in the changed interstitial tissue there were large hemorrhages. Another striking feature of these septums was the state of the lymph vessels, a great number of which were extremely dilated and packed with fibrin, polymorphonuclear cells and desquamated endothelial cells. Frequently these cell masses formed polyplike structures, seemingly starting from one part of the wall of the lymph vessel and protruding into the lumen. A few veins showed in their walls areas in which were small aggregations of polymorphonuclear cells and cells whose nuclei were markedly pyknotic. The endothelium lining the vessels near these areas was sometimes separated and pushed into the lumen by these cell aggregations. Here and there small veins were packed with many polymorphonuclear cells. A few small arteries

in the septums showed circumscribed periarteritis with accumulations of leukocytes and many pyknotic cells in the adventitia close to the media (fig. 2). Some arteries showed small infiltrations inside the media, likewise made up of leukocytes and pyknotic cells. Finally, in hematoxylin-eosin-stained sections there were rare small bluish areas consisting of closely approximated cells with so much karyorrhexis and pyknosis that they could not be identified. In slides stained with polychromic methylene blue these necrobiotic areas revealed great numbers of bacilli; elsewhere only occasional bacilli were found.

The lung parenchyma was for the most part atelectatic—being infiltrated here and there with many leukocytes. The bronchi were likewise involved, containing

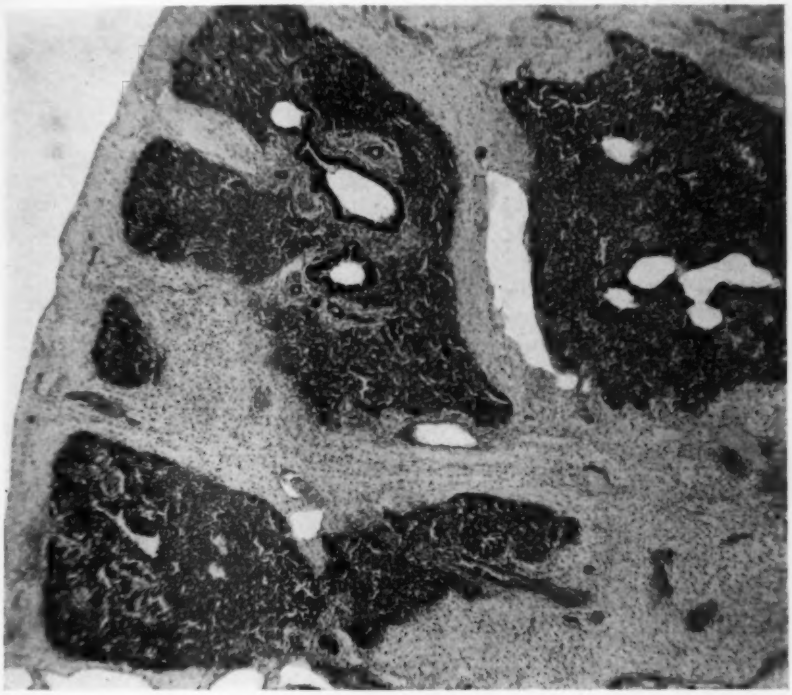


Fig. 1.—Interstitial pneumonia. Note the extreme widening of the septums due to serous exudate.

small, more or less well defined areas underneath the bronchial epithelium or, more often, closely bordering the cartilage rings. These areas were composed of many bacteria, polymorphonuclear cells and badly damaged shrunken cells which could not be identified. Other parts of the lung showed a somewhat different picture, the septums and the pleura not being so markedly widened by serous exudate but exhibiting extensive hemorrhagic extravasations. The neighboring partially atelectatic lung tissue was infiltrated with blood. These hemorrhages exceeded by far the inflammatory conditions.

The pleura showed a thin pseudomembrane consisting of leukocytes and fibrin, and the mesothelium underneath the pseudomembrane was extensively destroyed.

In the sinusoids, especially in the marginal sinusoid of the tracheobronchial lymph nodes, there was a great proliferation of reticuloendothelium, mixed with a few leukocytes and red blood cells. In many places there were karyorrhexis and pyknosis, obscuring the structure of the tissue within the sinusoids. The follicles were small and without germinal centers. The medullary cords did not show any special changes.

The myocardium presented no pathologic change; no fat was seen in sudan-stained slides. The epicardium was partly covered with a thin layer of fibrin and leukocytes and mononuclear cells. The subepicardial fibrous tissue was greatly involved. In places it showed marked thickening due to edematous infiltration and in addition extensive stripelike necrobiotic areas, where the structure was obscured.

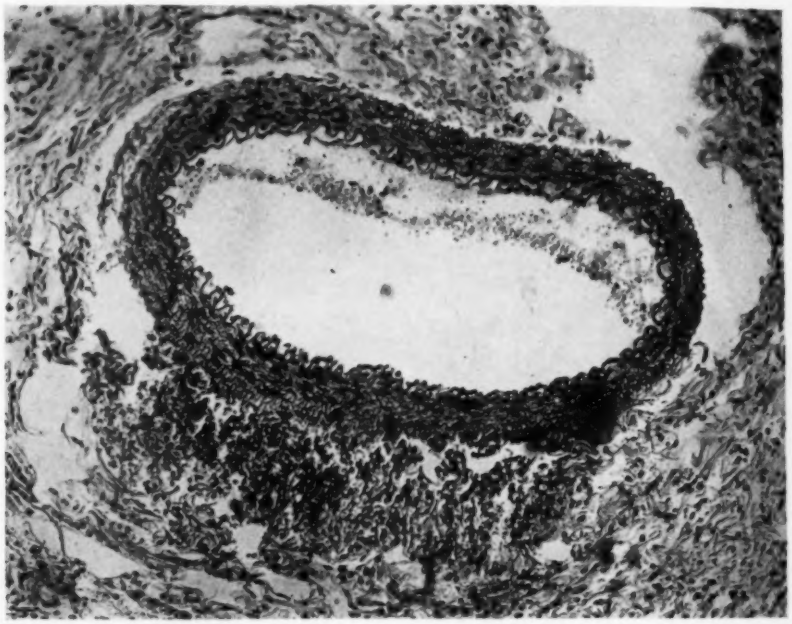


Fig. 2.—Circumscribed area of periarteritis in a septum of a lung, showing many polymorphonuclears and pyknotic cells.

The tissue was bluish with hematoxylin, and the nuclei were shrunken or broken. Some small arteries showed more or less marked infiltration of the media by shrunken and broken nuclei, which were closely approximated. These cells either formed circumscribed areas or involved the whole media; the intima and the lumen of the vessels were not altered. In other parts of the epicardium there were similar conditions but much less developed. The fibrous tissue of the fibrous annuli occasionally exhibited similar changes. The endocardium did not seem to be involved in this process.

In the loose periaortic tissue were many striplike areas showing the same necrobiotic changes as described in the heart and lungs.

The thymus showed marked interstitial inflammation, the interlobular septums being enlarged and in some places densely infiltrated by cells with round dark nuclei, simulating lymphocytes. More frequently, the septums were infiltrated by

nuclear fragments with thin irregular threadlike processes. In the capsule the inflammatory areas had a stretched-out, stripelike shape. Everywhere the necrobiotic changes were in evidence, the marking in these areas being obscured and the tissue showing a basophilic reaction with hematoxylin (fig. 3). Occasionally, the interstitial tissue revealed small well defined inflammatory areas simulating tiny nodules. Where necrobiotic changes did not obscure the structure there were lymphocytes, eosinophils and mononuclear wandering cells in various combinations. Occasionally, there were a few leukocytes within edematous areas. In some places the interstitium appeared normal. In contrast to the lung, there seemed to be no fibrinous exudate in the inflamed stroma. In sections stained with methylene blue there were many bacilli both within and without the necrobiotic areas. The thymic parenchyma did not exhibit any particular pathologic condition.

The brain did not show any abnormal condition in paraffin sections stained with hematoxylin and eosin. In the subarachnoid space, especially in the region

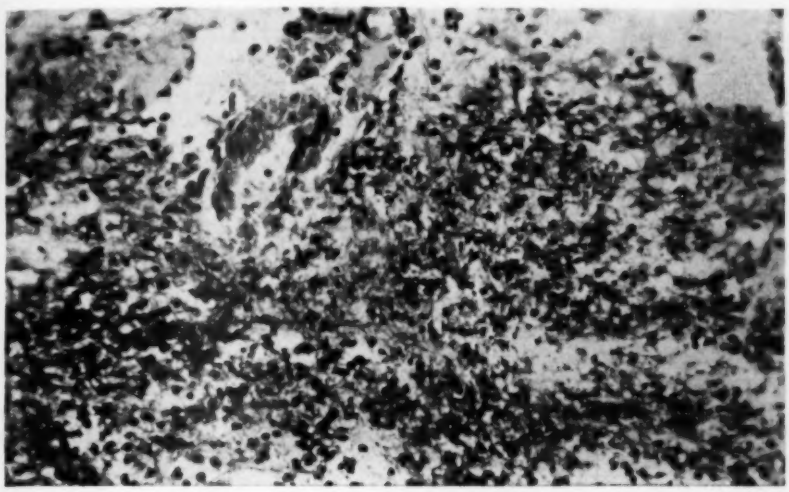


Fig. 3.—Inflammatory area in a septum of the thymus, showing marked necrobiosis with a great many cell fragments.

of the sylvian fissure and to a less extent over the frontal lobes there was extensive hemorrhage. The leptomeninges over the pons were infiltrated by lymphocytes, a few leukocytes and a few mononuclear wandering cells.

There was moderate hyperemia of the liver, with a few small blood cell areas; there was no fatty infiltration or inflammatory change of the parenchyma.

In the spleen there was hyperemia of the red pulp, in which were many eosinophils. The follicles contained only a few eosinophils.

The kidneys were hyperemic; the adrenals presented no special change.

The skin over the abdomen was normal.

COMMENT

As to the genesis of the B. pyocyaneus infection in this case, we first have to answer the question whether the infection was present when the baby was born or was acquired postnatally. As evidence for the first assumption, namely, that the baby was already sick when it was

born, are two significant facts: (1) The baby showed signs of severe sickness immediately after birth (rash, difficulty in breathing, cyanosis), and (2) the pathologic changes were so markedly developed that they must be considered older than the twenty hours which the baby lived.

As to how the fetus may have been infected in utero, there are various possibilities. Infection of a fetus may occur by transmission of a general infection of the mother from intervillous space through the chorion. It may be due to immediate inoculation of the organisms into the fetal tissue or into the placenta following injury in attempted abortion. A fetus may also become infected when amniotic fluid containing the organisms invades the respiratory tract, the digestive tract or the broken or unbroken skin or passes through a damaged amnion. Finally the fetus may be infected by passage of the organisms from the uterine wall directly through the placenta.

In our case there was no instrumental intervention prior to the delivery by which the fetus or the placenta could have been injured and infected, nor were there any signs that the amniotic fluid was infected. On the other hand, the mother showed signs of slight infection one day before delivery, consisting of chills, low fever and loose bowel movements. In addition, the feces of the mother one day post partum contained B. pyocyaneus, though she was clinically normal at this time. Furthermore, it was significant that the lochia was found sterile, an observation which hardly could have been made if the amniotic fluid had been infected from without. So it appears that a slight general infection of the mother by B. pyocyaneus was transmitted to the fetus, producing a fatal septicemia.

As to the infection of the mother, we may assume that slight enteritis occurred, with B. pyocyaneus invading the blood stream from the intestinal canal. The brief bacteremia was sufficient to permit infection of the fetus in utero through the chorion. Aside from the rare intrauterine B. pyocyaneus infection the case is remarkable because it shows that even in a healthy adult B. pyocyaneus may produce infection with bacteremia. This bacillus has been found in drinking water, especially in wells, in dust, in cotton waste and in other material. In a series of 3,000 stool examinations in cases in which typhoid, dysentery and cholera were suspected to be present, B. pyocyaneus was isolated thirteen times.[8] Unfortunately, it was not possible to find the source of infection of the mother, but in view of the facts we may assume that the infection occurred by intake of infected food or drink.

SUMMARY

Congenital B. pyocyaneus infection in a boy, who died twenty hours after birth, is reported. Cutaneous rash, dyspnea, cyanosis and leukopenia with related lymphocytosis were observed. The autopsy revealed septicemia due to B. pyocyaneus, with acute inflammatory and necrotic processes in the lungs, epicardium, leptomeninges and many other tissues. The source of the infection appeared to be a rather mild B. pyocyaneus infection of the mother just before and during delivery, marked by chills, fever, drowsiness, abdominal pain and diarrhea.

8. Bezi, S.: Beitr. z. path. Anat. u. z. allg. Path. **92**:41, 1933.

Notes and News

Appointments.—According to *Science,* Harry S. N. Greene, of the Rockefeller Institute of Medical Research, Princeton, N. J., has been appointed professor of pathology and surgery in Yale University.

D. Murray Angevine, assistant professor of pathology in the Cornell University School of Medicine, has been appointed pathologist and bacteriologist in the Alfred I. du Pont Institute of the Nemours Foundation at Wilmington, Del.

The directors of the Finney-Howell Research Foundation, Inc., 1211 Cathedral Street, Baltimore, have awarded annual fellowships to the following: Glenn Horner Algire, to work at the National Cancer Institute, Bethesda, Md.; Earl Leroy Green, to work at the Jackson Memorial Laboratory, Bar Harbor, Maine; Joseph Gilbert Hamilton, to work at the University of California Radiation Laboratory, Berkeley; Rose I. Shukoff, to work at the Glasgow Royal Cancer Hospital, Glasgow, Scotland. Fellowships were renewed for a year for Margaret Nast Lewis, Bernerd E. Kline, Julius Charles Abels, Alfred Marshak, John F. Menke and Paul C. Zamecnik. Fellowships carrying an annual stipend of $2,000 are awarded for the period of one year with the possibility of renewal up to three years, at the annual meeting of the directors, held near the end of February. Fellowships are awarded only for research into the causes and treatment of cancer. Applications must be made on the blank forms furnished by the secretary and must be filed in the office of the foundation *before* January 1 of each year.

Awards.—R. J. Williams, of the University of Texas, is the 1941 recipient of the Mead Johnson & Company $1,000 vitamin B complex award for "outstanding work in the discovery, isolation and identification of pantothenic acid."

The Walker Prize for Cancer Research College of Surgeons of England has been awarded to Dr. Peyton Rous, of the Rockefeller Institute for Medical Research.

Society News.—The American Association of Pathologists and Bacteriologists has elected officers as follows: president, S. R. Haythorn; vice president, P. R. Cannon; secretary, H. T. Karsner; treasurer, Alan R. Moritz. The next meeting of the association will be held in St. Louis, April 2 and 3, 1942. At this meeting there will be a symposium on neoplasms of the endocrine glands.

In connection with the celebration of the seventy-fifth anniversary of the University of New Hampshire, the American Association for the Advancement of Science will hold a meeting at Durham, N. H., from June 23 to 28. The association will hold a meeting in Chicago September 22 to 27 in connection with the celebration of the fiftieth anniversary of the University of Chicago.

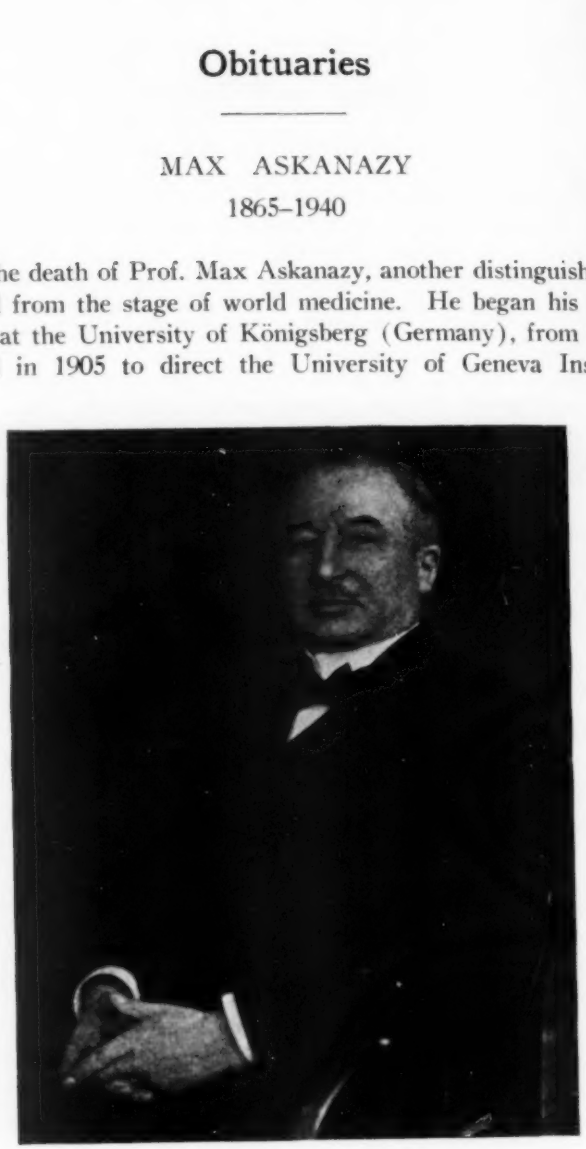
Permanent Science Fund.—Income from this fund, according to agreement and declaration of trust, shall be applied by the American Academy of Arts and Sciences to such scientific research as shall be selected. Applications for grants under this trust are considered by the committee of the academy on stated dates. To be considered at the next meeting of the committee, applications must be in the hands of the chairman of the committee, John W. M. Bunker, Massachusetts Institute of Technology, Cambridge, Mass., on or before Oct. 1, 1941.

Obituaries

MAX ASKANAZY

1865–1940

With the death of Prof. Max Askanazy, another distinguished figure has passed from the stage of world medicine. He began his career in pathology at the University of Königsberg (Germany), from which he was called in 1905 to direct the University of Geneva Institute of



MAX ASKANAZY

1865–1940

Pathology. He occupied this post until his retirement in the spring of 1939. His curiosity concerning the phenomena of human disease led to the exploration of various fields. His contributions ranged through parasitology, hematology, immunology, diseases of the thyroid, parathyroid, adrenal, pineal gland and hypophysis, tuberculosis of nerve struc-

tures, arteriosclerosis, peptic ulcer and cirrhosis, to the broad field of cancer research which during later decades was the realm of his most productive activity. Though 75 years of age at the time of death, in October 1940, he was youthful in spirit, and his keen faculties were unimpaired. By an ironic stroke of fate, he died of the disease he had studied most intently.

Askanazy was among the first to sense the relationship of the parathyroid gland to calcium metabolism and to show the effects of parathyroid hyperactivity on skeletal structure. His report on parasitic infestation (Opisthorchis felineus) as a cause of hepatic carcinoma was one of the earliest observations on the relation of parasites to carcinogenesis. His all-embracing interest in pathogenesis combined with a highly retentive memory contributed to an encyclopedic command of medical lore. The quality of his work received notable recognition in the form of various honors, among which were the Marcel Benoist Prize and a distinguished honor awarded by the city of Geneva in 1935.

Askanazy sensed the role of environmental influences in processes of disease and sought means for evaluating these. He initiated the organization of the International Society for Geographic Pathology, which had for its purpose the assembling of data on such factors, and he supplied the motivating energy which extended this organization into thirty-nine countries.

The Institute of Pathology at Geneva University became known for its friendly atmosphere because medical visitors, whether pathologists or others, were accorded an unusual type of genuine hospitality. American physicians will remember Professor Askanazy for three outstanding traits of character: his versatility and the accuracy of his memory, his quiet unassuming modesty of demeanor and the innate charm of his personality. Workers in fields of medical science, particularly in that of pathology, will regret keenly the passing of an eminent colleague, and those who were privileged to know him personally will grieve the loss of a genial friend.

VIRGIL H. MOON.

Book Reviews

Death and Dentistry. Martin H. Fischer, M.D., professor of physiology, University of Cincinnati. Cloth. Pp. 242, with 62 illustrations. Price $5. Springfield, Ill.: Charles C. Thomas, Publisher, 1940.

This book, as might be surmised from the dramatic title, is an impassioned presentation of the doctrine of focal infection in its most extreme form. As an illustration, the preface may be quoted directly: ". . . . the concept of the focal infection as first preached by Frank Billings and crystallized into scientific dogma by his pupils, marks the greatest advance and the greatest good that have come to a suffering world in this century."

The author's thesis is set forth in characteristically "literary" style in the concluding paragraph of his book: "You cannot mean, it has been asked of us, that only infection is responsible for all heart disease, the blue-sky nephritides, the ulcerations of the gastro-intestinal tract not due to immediate injury, the spotty and 'idiopathic' forms of nervous disease, vascular disease with the tribulations that hang from it as more heart disease, 'chronic' nephritis and the 'old age' motor and sensory crack-ups of the central nervous system? that every acute or chronic appendicitis, gastric ulcer, cholecystitis, iritis, glaucoma, and pyelo-nephritis, cystitis, prostatitis, orchitis, oöphoritis (when not of ascending origin) was induced because of metastatic blood-borne infection? that similar mechanisms must be called to mind in half the eczemas and all the dysendocrinisms and diseases of the mind not 'inherited'? and that arrest of existent, or freedom from future and renewed attack is assured only by the discovery and surgical eradication of a focus nearly always resident in tooth (or tonsil)? These questions need diplomatic answer. We quote the words of an international document: That, Gentlemen, is exactly what we mean."

This thesis is based in large part on the familiar work of E. C. Rosenow. Streptococci, gaining a portal of entry in teeth or tonsils, give rise to bacterial emboli which are in turn the cause of the diseases cited. The variety of disease processes is explained by elective or specific localization of different strains of organisms. Elimination of primary foci cures systemic disease, as illustrated by numerous case histories, since metastatic foci do not usually become chronic.

Three chapters at the beginning of the book and three at the end are concerned with the medical aspects of the problem, and the intervening five chapters, with the theory and practice of dentistry. In both fields the author shows himself to be an individualist with scant respect for the usually accepted doctrines of either medicine or dentistry. Among his unorthodox statements may be listed his comment that in lobar pneumonia "the tonsils are invariably involved, and we have never seen a lobar pneumonia in a properly tonsillectomized individual" and his comments on malignant overgrowths—"The activity of a microorganism producing local change (like the elaboration of an anthracene-like body) could explain it all. . . . We have never observed a malignancy, even in the young, in individuals not possessed of obviously bad teeth and tonsils."

Concerning the teeth he states that "enamel is sensitive and if cracked (without infection!) heals." On page 78 he speaks of "new cells that have formed in the dentine to drive its fibrils apart" during the progress of dental caries. (His figure 29, however, shows only the usual picture of carious dentine containing clumps of bacteria and unidentifiable organic matter.) He apparently believes that all sclerosed dentin, white in ground section, is infected. Furthermore, he states that after extraction of a tooth the alveolar process is deprived of its blood supply and that the proper operation is to remove the process to the tip of the root (fig. 43, opposite p. 87). Most startling of all, to a dentist, is his statement "We never place fillings until in the weeks following an excavation, all pain in the area

has disappeared. And then it is done only because a school board compels, a dental association demands, or the patient, because young and mistaught, insists."

These excerpts are no more extreme than many others which might have been selected. There can be no doubt that infection in and about the teeth may cause metastatic infection or that many patients have been benefited by the extraction of diseased teeth and the curettage of the surrounding alveolar bone. From this it is a long step to the hypothesis that all or nearly all disease of questionable or unknown origin is caused by focal infection of dental origin. This hypothesis has been losing ground for twenty years, and its proponents, if they would rehabilitate it, should offer bacteriologic and pathologic as well as clinical evidence for it. The book purports to do just this, but the evidence presented is not adequate to justify the author's conclusions.

The book is well bound and attractively printed. The bibliography, except for previous publications by the author, is placed at the foot of the pages throughout the volume and is limited almost entirely to proponents of the theory of focal infection. There is an index of names and one of subjects.

Angina Pectoris. Nerve Pathways, Physiology, Symptomatology and Treatment. H. R. Miller, M.D., Attending Physician, Sydenham Hospital, and Associate Attending Physician, Montefiore Hospital, New York. Cloth. Pp. 275, with 39 illustrations. Price $3.25. Baltimore: William Wood & Company, 1939.

The most important part of this book is contained in the 114 pages in chapters 1 to 6. These are concerned with "the anatomical pathways for the transmission of cardio-aortic pain," and "the distribution or reference of anginal (cardio-aortic) pain." These pages are based on anatomic studies made with the collaboration of Dr. L. Vosburgh Lyons, of the Neurological Institute of Columbia University. The many drawings show that these studies were prolonged and careful. Some of the drawings are schematic. There are helpful legends with the figures. This work is really the justification for the book as a whole. On it the author largely bases his discussion of the clinical features and the treatment of angina. This topic, treatment, takes up about one quarter of the volume. One may question the wisdom of including in a volume of this size the results of the laboratory and bibliographic research dealing with the fundamental processes concerned in pain in general and in particular that of angina pectoris, and the well known clinical features of this condition. Physiologists and neurologists will be interested in the anatomic charts and may be inclined to ask for confirmation through the investigations of others. Some of the clinical manifestations will not attract them. Clinicians will, one fears, be considerably bewildered in trying to understand the drawings. They may, however, be glad to have the author's comments on the rationale of the surgical treatment. On the whole, one must applaud this somewhat venturesome undertaking. Any contribution tending to solve the complicated mystery of the syndrome called angina pectoris should be welcomed by anatomists, physiologists, pathologists and clinicians.

Étude morphologique et biologique sur les flagellés intestinaux parasites de murides. Étude comparative des flagellés du cobaye. Annales de l'Universite de Lyon. Troisième série—Médicine—Fasicule 1. Léon Morénas. Paper. Pp. 234, with 24 illustrations. Price 60 francs. Paris: Masson & Cie, 1938.

A brief preface introduces the subject, which has a literary history of over a hundred years. The importance of the flagellates goes beyond the scope of comparative parasitology and veterinary medicine, particularly since the recent interest in the infection of man with intestinal flagellates. The book is divided into two large parts. The first deals with the technic of study and with the morphology of the flagellates found in the intestines of a variety of species of Murides and of

guinea pigs. These flagellates are divided into protomonadids, with Trichomonas as the main representative, and diplomonadids, which include Giardia. The literature and a minute description of each protozoon type are included, illustrated with 12 schematic drawings. The second and last larger section of the book is devoted to biologic considerations. The presentation of each of the problems is divided into two portions; the first discusses the previous attempts to solve them and the results, while the second presents the investigations of the author. Here are some of the "high spots": Domestication enhances infection with flagellates; such infection is relatively rare in animals living in natural conditions, and, if present, it is usually of the monoparasitic type instead of the polyparasitic type of the domesticated laboratory animal. The flagellates related to Trichomonas are regular inhabitants of the contents of the cecum, while Giardia dwells in the mucous secretion of the upper part of the small intestine. Parasites do not appear in the intestines of young animals until they are weaned. Animals free from parasites were bred by isolating them from their mothers. There was no apparent relation between the hydrogen ion concentration of the intestinal contents and the parasites. High protein diet had an inhibitory effect on some flagellates. Vitamin A deficiency led to a decrease and even to a disappearance of flagellates, while vitamin C deficiency had no such effect in the guinea pig. Predominance of a gram-positive bacterial flora was found incompatible with the existence of flagellates in the cecum; there was also noted a similar incompatibility with infestation by worms. The attempts to culture flagellates, the modes of infection and the host-parasite relations are the subjects of the last three chapters. A 14 page bibliography concludes this clearly written monograph, which should be of great interest to parasitologists.

Debatable Tumours in Human and Animal Pathology. W. F. Harvey, E. K. Dawson and J. R. M. Inness. From the Research Laboratory of the Royal College of Physicians, Edinburgh, Scotland, the Cancer Organization of Edinburgh and South-East Scotland, and the Institute of Animal Pathology, University of Cambridge. Price, 10 shillings 6 pence. Pp. 124, with 40 plates. Edinburgh and London: Oliver & Boyd, 1940.

This book, the first of a projected series of cancer research memoirs, is made up of revised articles originally published in the *Edinburgh Medical Journal*. It deals with the results of a comprehensive and intensive morphologic study of the following tumors: seminoma, granulosa cell tumor of the ovary, mixed tumors of salivary glands, melanoma, lymphoepithelioma, giant cell tumor of bone, endothelioma, lymphosarcoma, meningioma. More than 15,000 examples of these tumors were studied. In the main, of course, these were tumors of human beings, but references are also made to the study in animals of all the tumors except lymphoepithelioma and meningioma. The authors noted that in animals intracranial tumors are remarkably infrequent.

The descriptions and discussions are clear and compact and lead to definite summaries. Each tumor is abundantly illustrated, and notes in the legends, clinical and otherwise, amplify the text proper. There are 40 plates, and each plate carries from 8 to 15 or more figures. The illustrations are models of skill in microscopic technic, in photography and in artistic and economic arrangement for reproduction. The figures show how satisfactorily microscopic structure can be illustrated by small fields. There is a selected list of "directional literature" for each tumor.

The effort of the authors to trace each tumor back to the cell, or cells, of its origin definitely clarifies the understanding of its nature and of its varieties. This clarification is effected particularly with regard to the most "debatable" tumors of the series, namely, mixed tumors of salivary glands, melanoma, lymphoepithelioma, endothelioma and lymphosarcoma. (In the discussion of the cells in melanoma there occurs this sentence [p. 29]: "The difficulty remains how to distinguish the melanocyte from the melanophage other than by defining the former

as a cell-forming pigment, the latter as cell-ingesting pigment." Obviously pigment-forming cell and pigment-ingesting cell were meant.) The book will be of great interest and help to all students of tumors.

La tuberculose du cobaye. J. Paraf. Paper. Pp. 132. Price 30 francs. Paris: Masson & Cie, 1939.

The eminent role that the guinea pig plays in the clinical diagnosis of tuberculosis, as well as in research, makes the study of the anatomy, physiology and pathology of that animal important. This monograph, one of the few on the subject, will arouse considerable interest. On 119 pages of text Paraf has condensed a myriad of facts that are scattered throughout the literature and included some of his own observations, previously published. The first section deals with the breeding of the animals and contains many valuable details. It deals also with the anatomy and the growth, and includes tables of weights and sizes of organs at different ages. The measuring of the temperature, the operative methods, the anesthesia, the different routes for inoculation of suspected material and the autopsy technic are then discussed. In the description of the spontaneous diseases is a clear differentiation of pseudotuberculosis from true tuberculosis. The largest chapter, 32 pages, is given to induced tuberculosis. Here are considered the influence of different modes of inoculation on the course of the disease and on the distribution and appearance of the lesions; the differences due to the use of human, of bovine and of avian tubercle bacilli, and of acid-fast bacilli isolated from cold-blooded animals and from other sources; the effects of the quantity of the bacilli and their quality (low virulence or killed organisms); the lesions produced by filtrates of cultures and by isolated fractions of bacilli. The final chapters take up the development of hypersensitiveness to tuberculin, the changes in the blood picture, weight and size of tuberculous guinea pigs, the manifestations of reinfection and the influence of different portals of entry, the recognition of tuberculosis in the diseased animals and the attempts to immunize against tuberculosis, with particular emphasis on BCG. The monograph closes with a comparison of tuberculosis in man and in the guinea pig. The relations between the quantity of human tubercle bacilli, the route of inoculation, the clinical picture of the disease, the duration and the pathologic aspects of the lesions in the guinea pig are instructively presented in a table.

It is apparent from the review of the contents that the book holds a large store of information. The material is well organized, and it is clearly and economically presented. The only fault is a rather incomplete biblography, covering about 3 pages. A considerable number of authors are quoted in the text whose names are not listed in the bibliography. Apart from this drawback, the book can be warmly recommended.

Books Received

THE MEDICAL ASPECT OF BOXING. Ernst Jokl, M.D., head of the department of physical education, Witwatersrand Technical College, Johannesburg, South Africa; consultant, Medical Aspect of Physical Education S.A. Defence Force. Pp. 251, with 55 figures. Pretoria, South Africa: J. L. Van Schaik, Limited, 1941.

ORAL PATHOLOGY: A HISTOLOGICAL, ROENTGENOLOGICAL AND CLINICAL STUDY OF THE DISEASES OF THE TEETH, JAWS AND MOUTH. Kurt H. Thoma, D.M.D., professor of oral surgery, and Charles A. Brackett, professor of oral pathology, Harvard University. Pp. 1306, with 1370 illustrations, including 137 in color. Price $15. St. Louis: C. V. Mosby Company, 1941.

BIOLOGICAL SYMPOSIA, A SERIES OF VOLUMES DEVOTED TO CURRENT SYMPOSIA IN THE FIELD OF BIOLOGY. Edited by Jacques Cattell, editor of the American Naturalist and American Men of Science. Volume III: Muscle, edited by Wallace O. Fenn, professor of physiology, University of Rochester School of Medicine and Denistry, Rochester, N. Y. Pp. 370. Price $3.50. Lancaster, Pa.: Jacques Cattell Press, 1941.

BIOLOGY OF THE LABORATORY MOUSE. By the staff of the Roscoe B. Jackson Memorial Laboratory: Clarence C. Little, director; George D. Snell, editor; J. J. Bittner, A. M. Cloudman, E. Fekete, W. E. Heston, W. L. Russell, G. W. Woolley. With a chapter on infectious diseases of mice by J. H. Dingle, Harvard Medical School Pp. 497. Price $7. Philadelphia: The Blakiston Company, 1941.

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